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Werber et al.

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(45) **Date of Patent: Sep. 15, 2015**

(54) **ACELLULAR AMNION DERIVED THERAPEUTIC COMPOSITIONS**

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(22) Filed: **Jan. 9, 2015**

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(51) **Int. Cl.**

A61K 35/50	(2015.01)
A61K 9/19	(2006.01)
A61K 35/35	(2015.01)
A61K 35/44	(2015.01)
A61K 35/28	(2015.01)
A61M 37/00	(2006.01)

(52) **U.S. Cl.**
CPC . ***A61K 35/50*** (2013.01); ***A61K 9/19*** (2013.01); ***A61K 35/28*** (2013.01); ***A61K 35/35*** (2013.01); ***A61K 35/44*** (2013.01); ***A61M 37/0015*** (2013.01); *A61M 2037/0023* (2013.01); *A61M 2037/0061* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(74) *Attorney, Agent, or Firm* — Invention To Patent Services; Alex Hobson

(57) **ABSTRACT**

Acellular amnion derived therapeutic compositions are described having a number of various compositional embodiments. An acellular amnion derived therapeutic composition has essentially no live or active amniotic stems cells. The amniotic stem cells may be destroyed, and the cells and cell debris may be removed from the acellular amnion derived therapeutic composition. An acellular amnion derived therapeutic composition may comprise micronized amniotic membrane particles, and/or amniotic fluid. An acellular amnion derived therapeutic composition may be a dispersion of micronized amniotic membrane combined with a fluid, such as plasma, saline, amniotic fluid, combinations thereof and the like. An acellular amnion derived therapeutic composition may be combined with a matrix component to form a composite. An acellular amnion derived therapeutic composition may be used in conjunction with a composition comprising viable cells, such as stem cells.

30 Claims, 17 Drawing Sheets

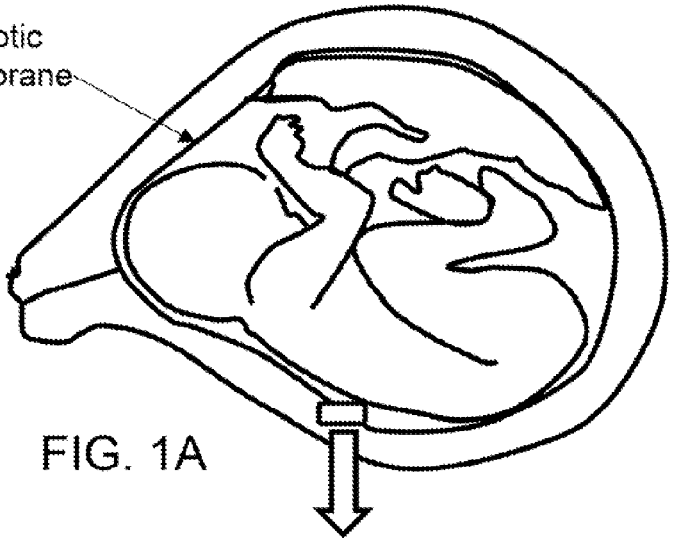

Amniotic
Membrane

FIG. 1A

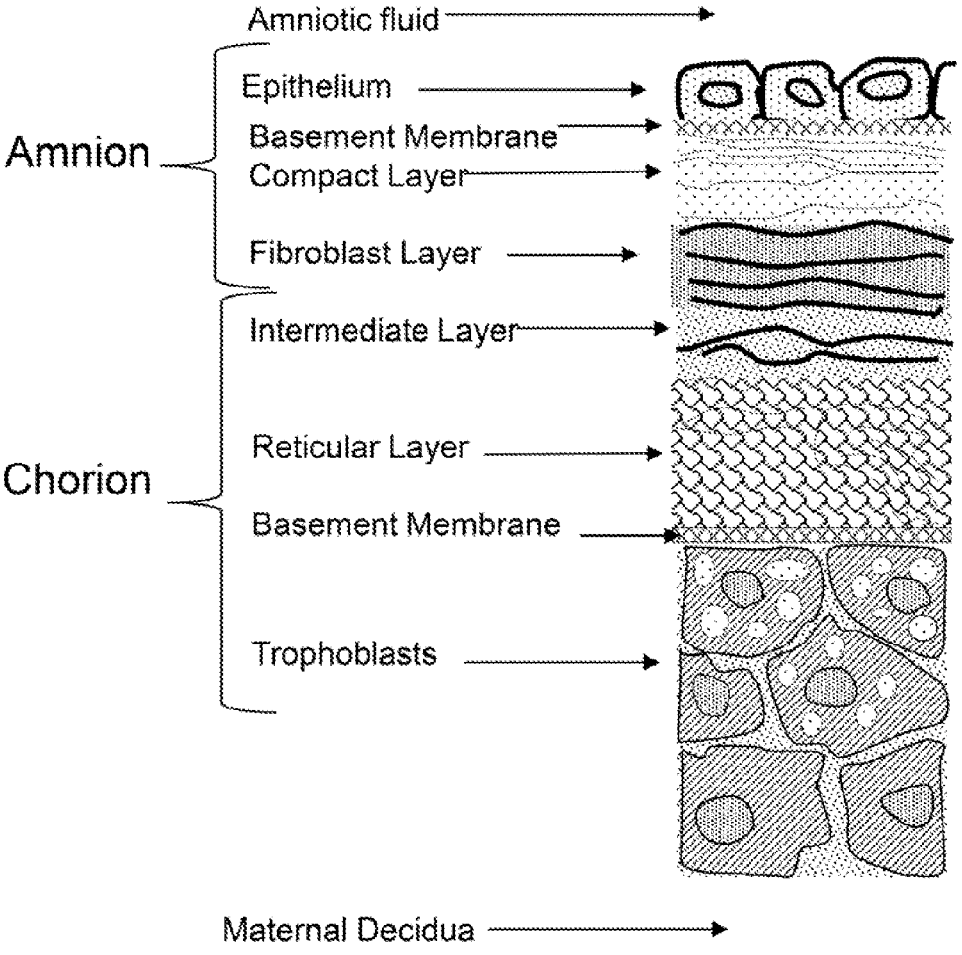
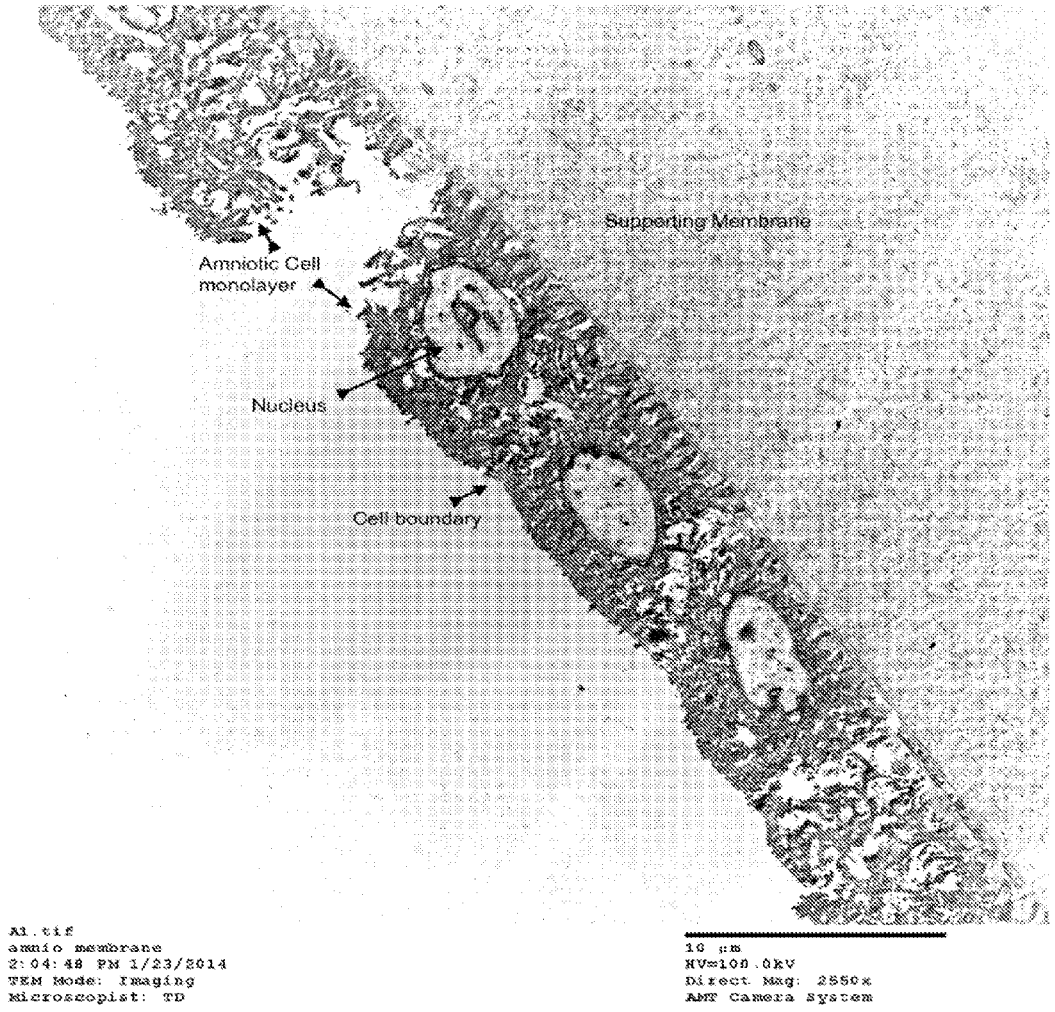

Amniotic fluid
Amnion
Epithelium
Basement Membrane
Compact Layer
Fibroblast Layer
Chorion
Intermediate Layer
Reticular Layer
Basement Membrane
Trophoblasts
Maternal Decidua

FIG. 1B


Supporting Membrane
Amniotic Cell monolayer
Nucleus
Cell boundary
A1.tif
amnio membrane
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Microscopist: TD
10 µm
HV=100.0kV
Direct Mag: 2550x
AMT Camera System

FIG. 2A

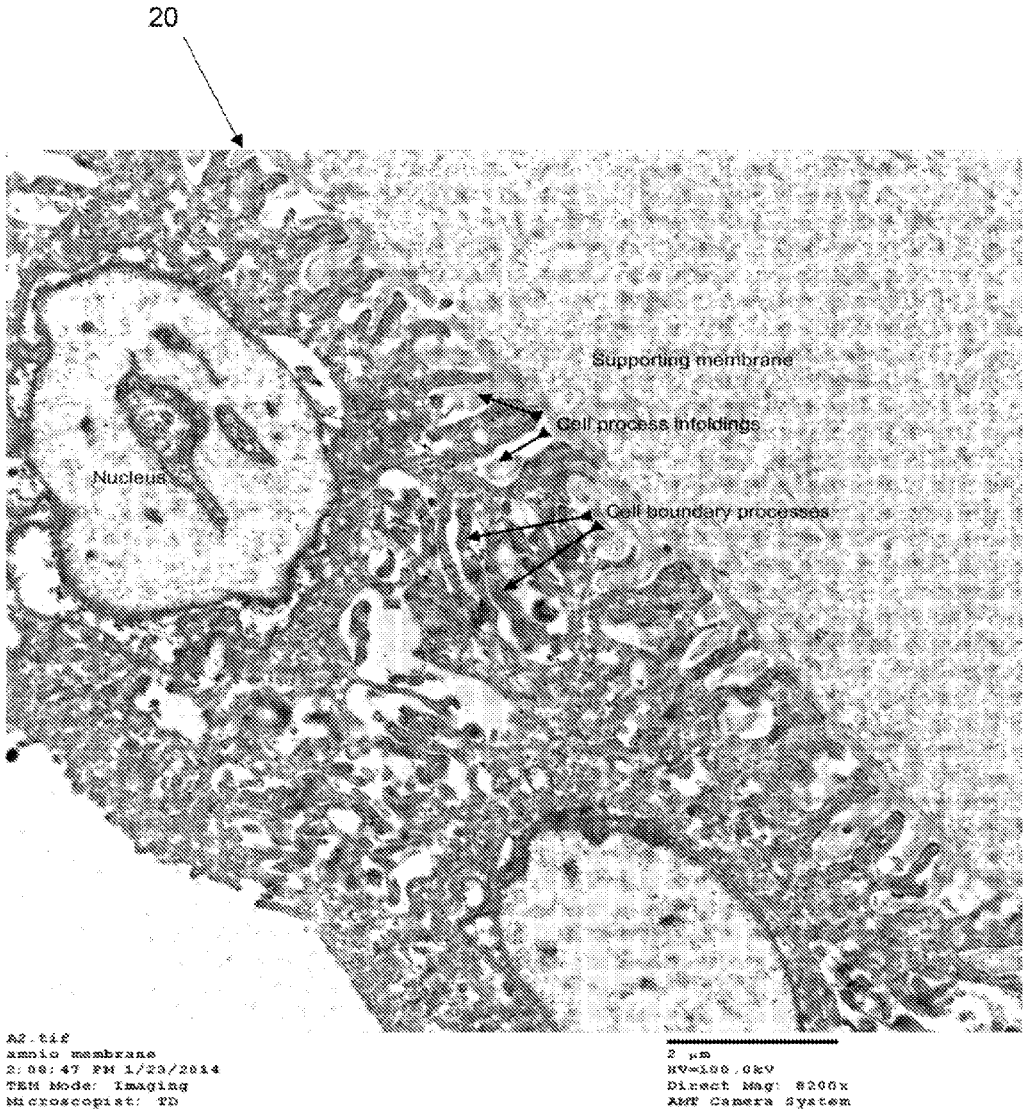
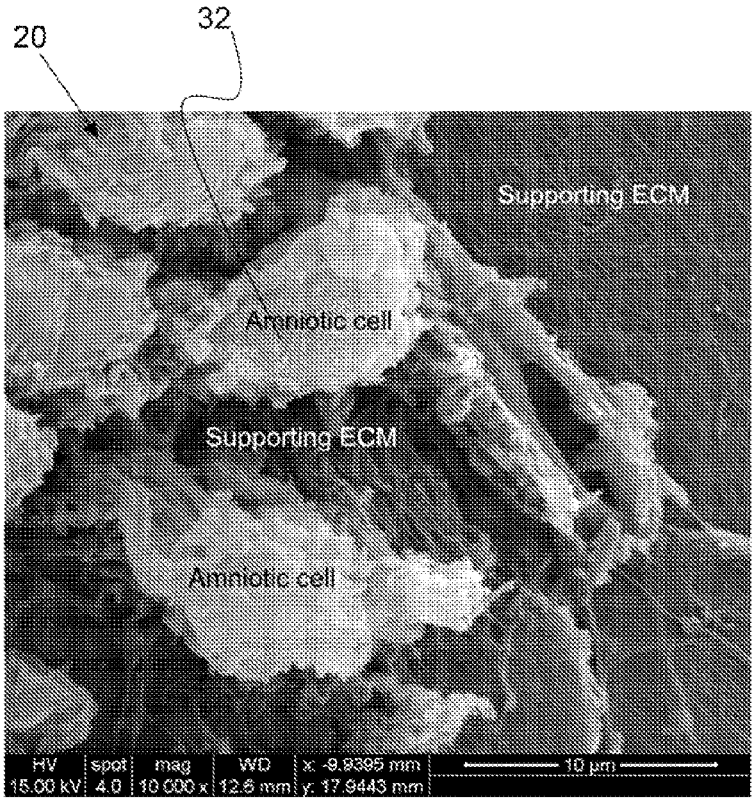

20
Supporting membrane
Cell process infoldings
Nucleus
Cell boundary processes
A2.tif
amnio membrane
2:08:47 PM 1/23/2014
TEM Mode: Imaging
Microscopist: TD
2 µm
HV=120.0kV
Direct Mag: 8200x
AMT Camera System

FIG.2B


20
32
Supporting ECM
Amniotic cell
Supporting ECM
Amniotic cell
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15.00 kV 4.0 10 000 x 12.6 mm y: 17.9443 mm
10 µm

FIG. 3A

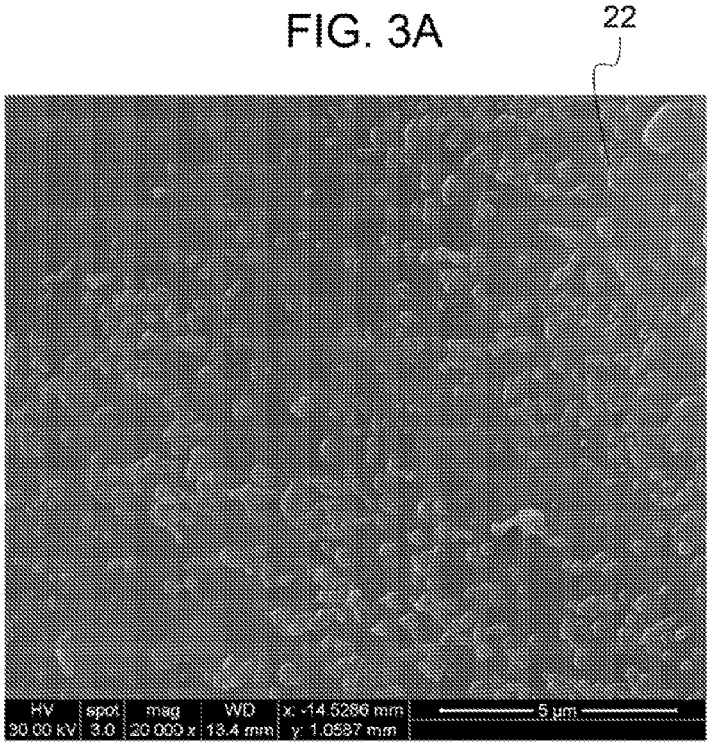
22
HV spot mag WD x: -14.5286 mm
20.00 kV 3.0 20 000 x 13.4 mm y: 1.0587 mm
5 µm

FIG. 3B

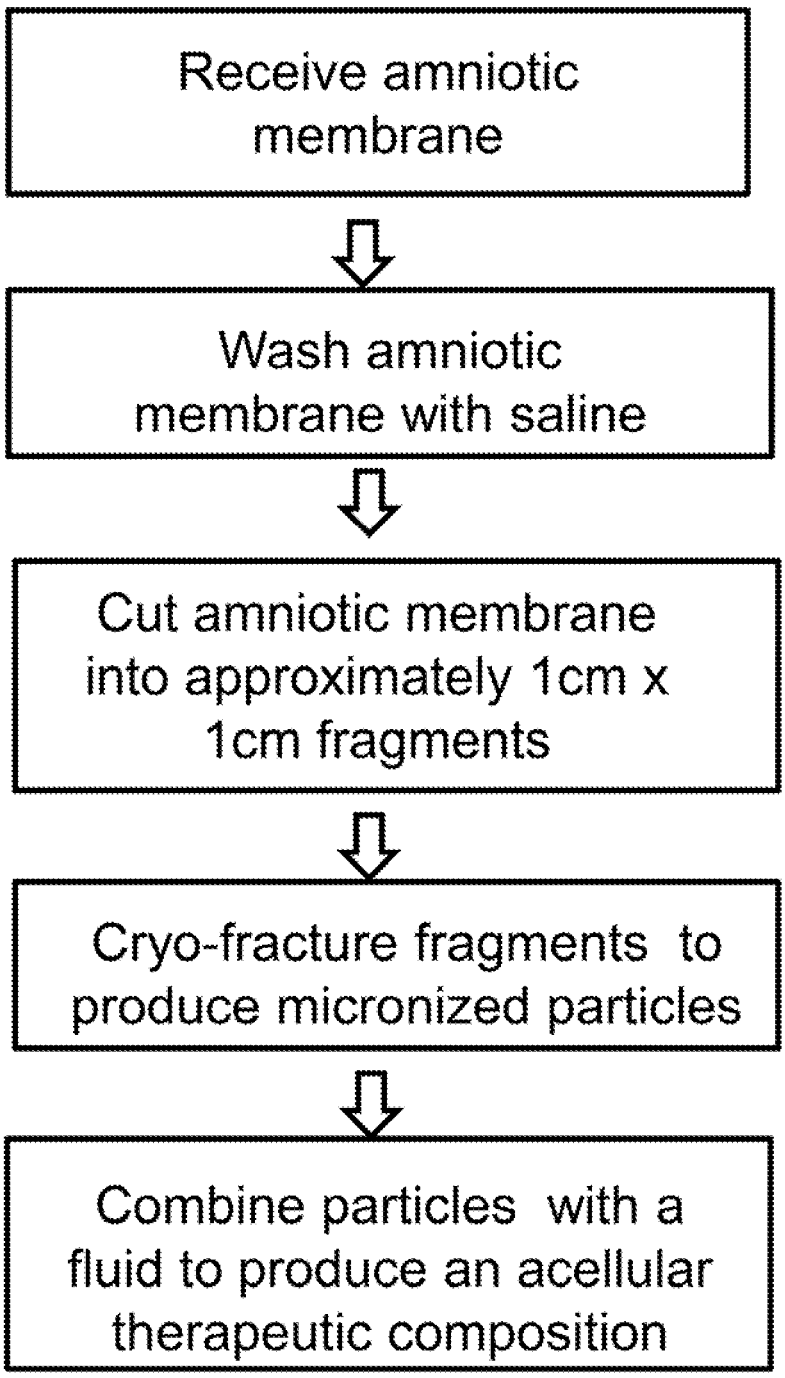
Receive amniotic membrane
Wash amniotic membrane with saline
Cut amniotic membrane into approximately 1cm x 1cm fragments
Cryo-fracture fragments to produce micronized particles
Combine particles with a fluid to produce an acellular therapeutic composition

FIG. 4

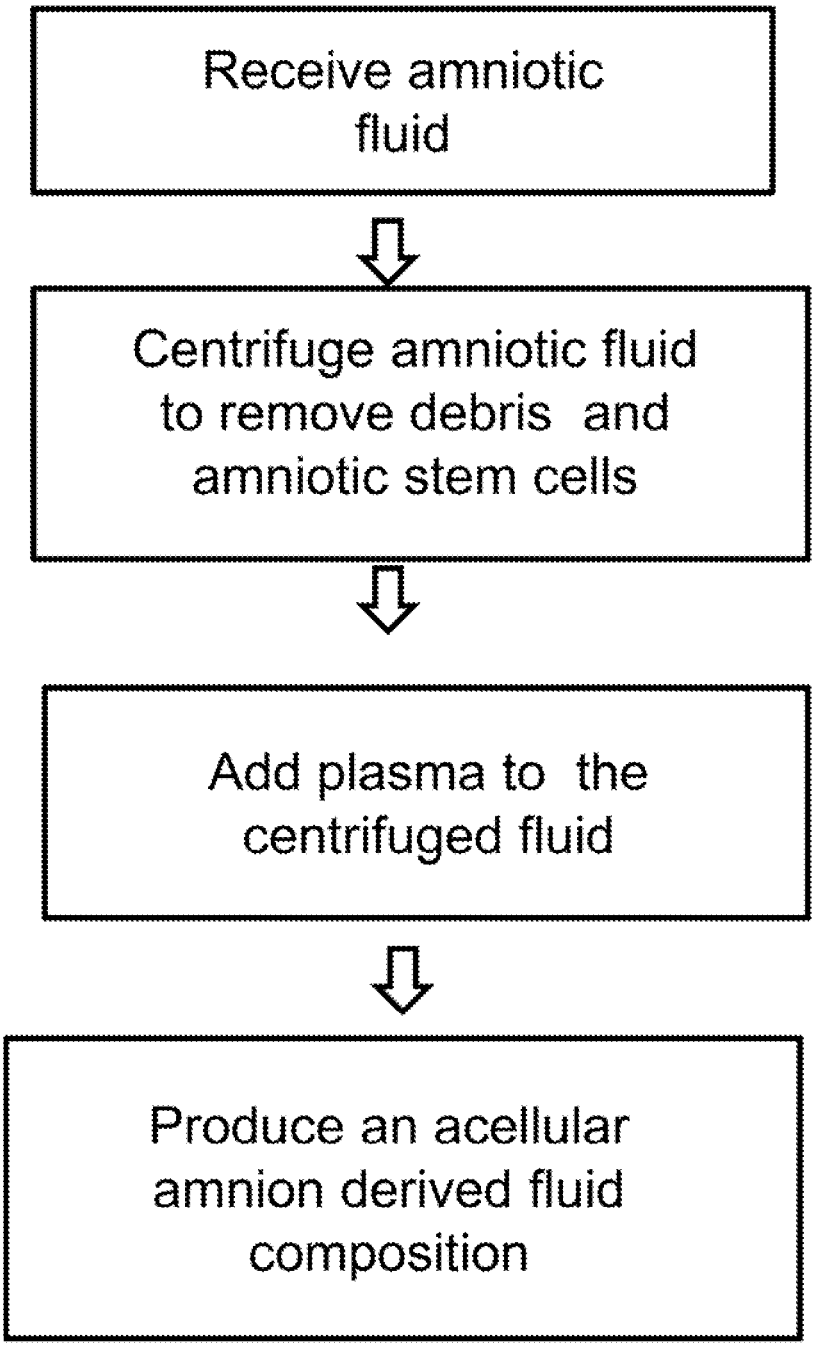
Receive amniotic fluid
Centrifuge amniotic fluid to remove debris and amniotic stem cells
Add plasma to the centrifuged fluid
Produce an acellular amnion derived fluid composition

FIG. 5

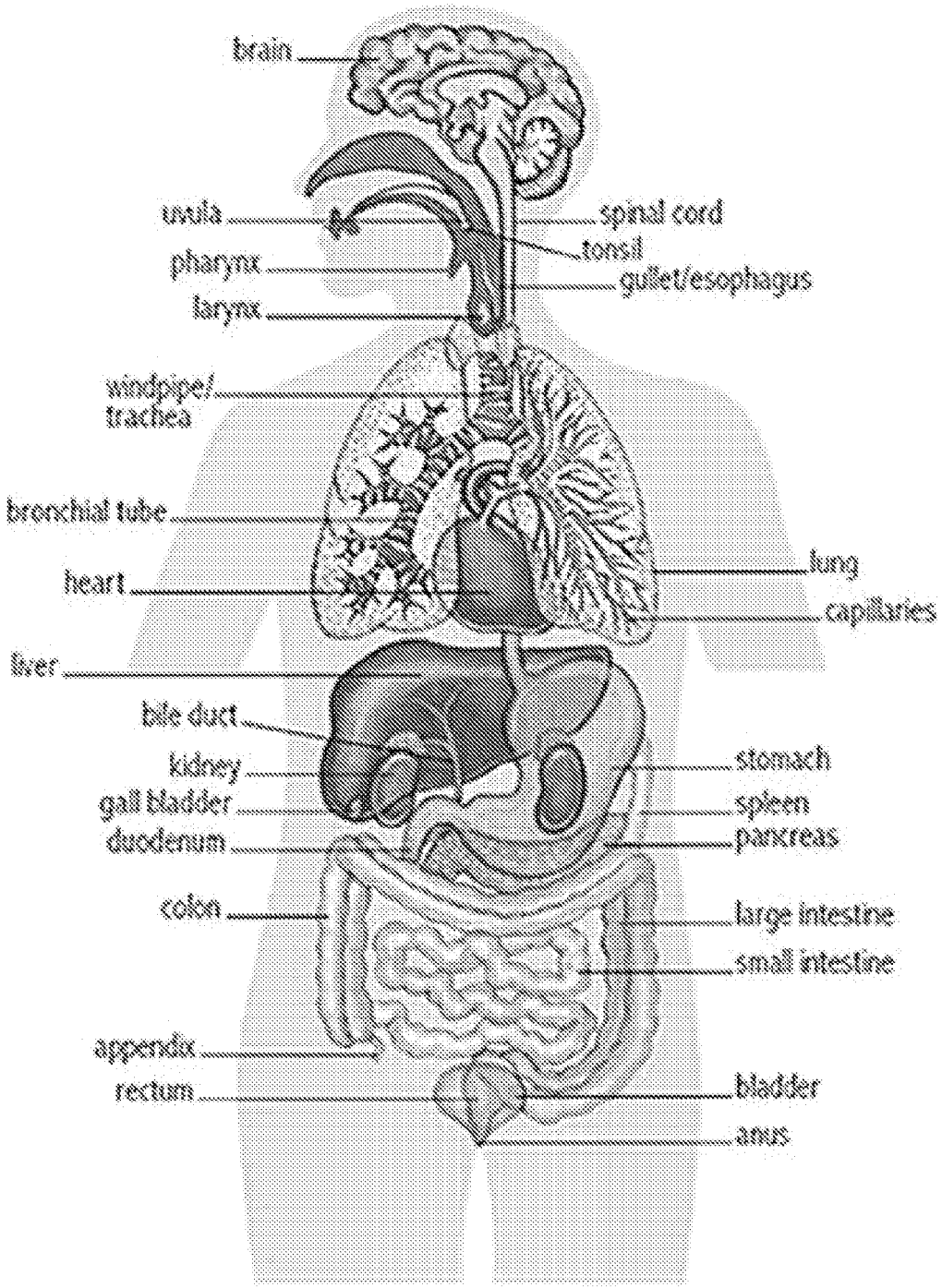
brain
uvula
pharynx
larynx
windpipe/
trachea
bronchial tube
heart
liver
bile duct
kidney
gall bladder
duodenum
colon
appendix
rectum
spinal cord
tonsil
gullet/esophagus
lung
capillaries
stomach
spleen
pancreas
large intestine
small intestine
bladder
anus

FIG. 6

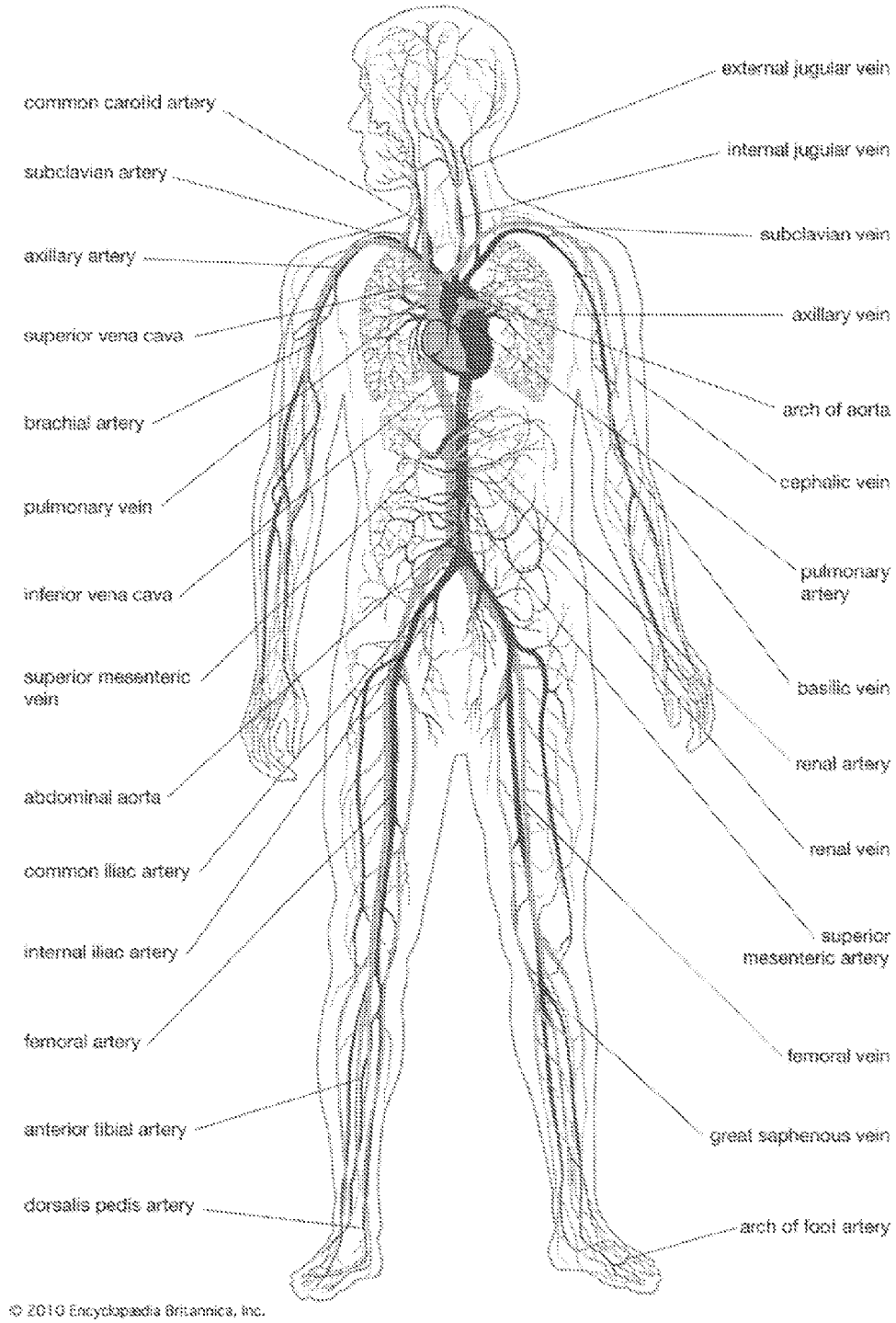
common carotid artery
subclavian artery
axillary artery
superior vena cava
brachial artery
pulmonary vein
inferior vena cava
superior mesenteric vein
abdominal aorta
common iliac artery
internal iliac artery
femoral artery
anterior tibial artery
dorsalis pedis artery
external jugular vein
internal jugular vein
subclavian vein
axillary vein
arch of aorta
cephalic vein
pulmonary artery
basilic vein
renal artery
renal vein
superior mesenteric artery
femoral vein
great saphenous vein
arch of foot artery
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FIG. 7

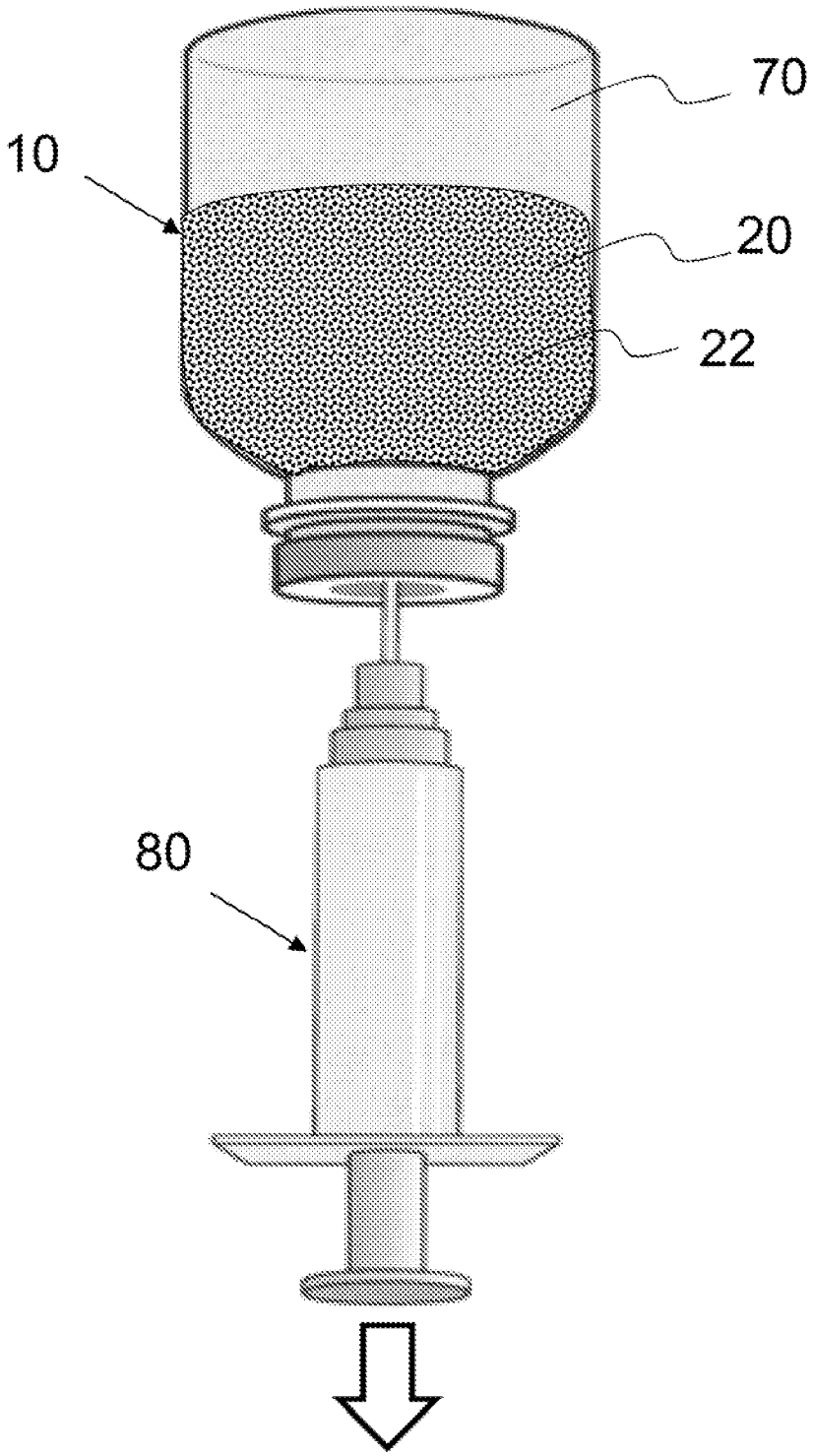

FIG. 8

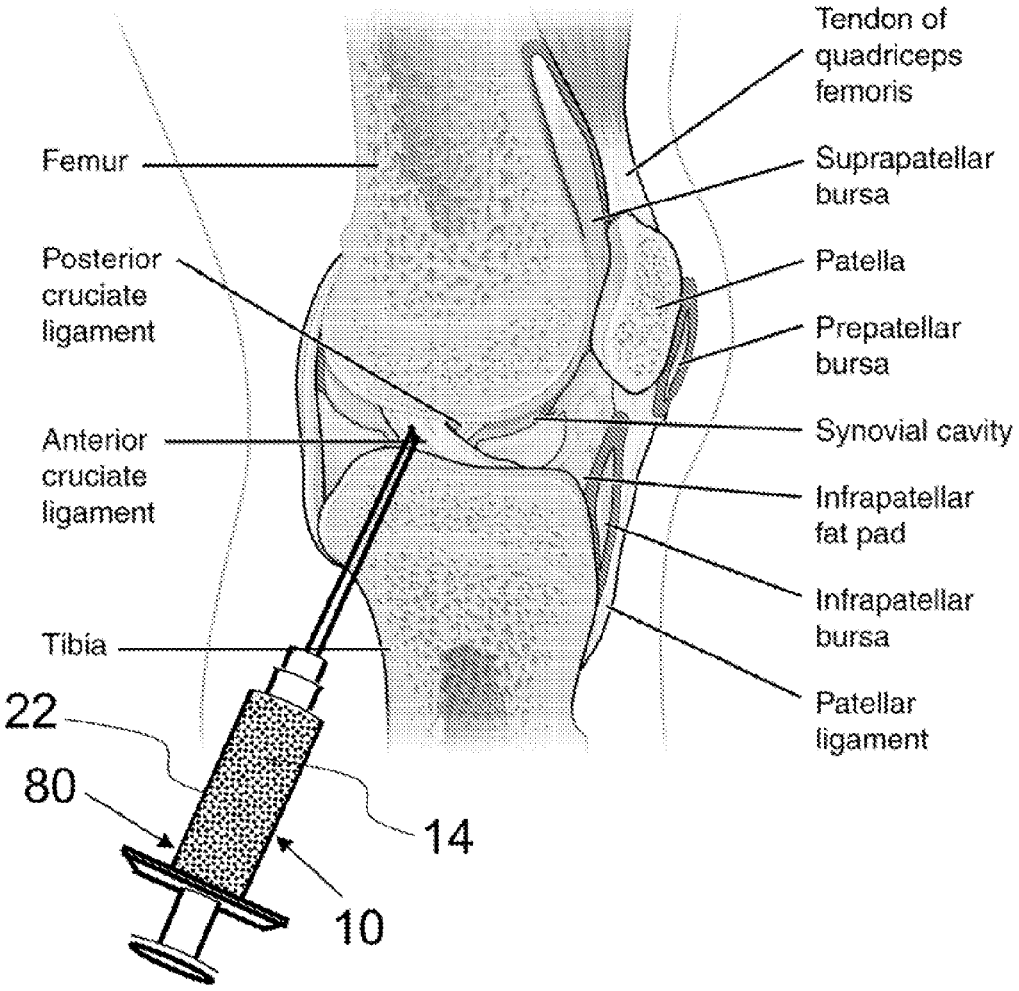
Tendon of quadriceps femoris
Femur
Suprapatellar bursa
Posterior cruciate ligament
Patella
Prepatellar bursa
Anterior cruciate ligament
Synovial cavity
Infrapatellar fat pad
Infrapatellar bursa
Tibia
Patellar ligament
22
80
14
10

FIG. 9

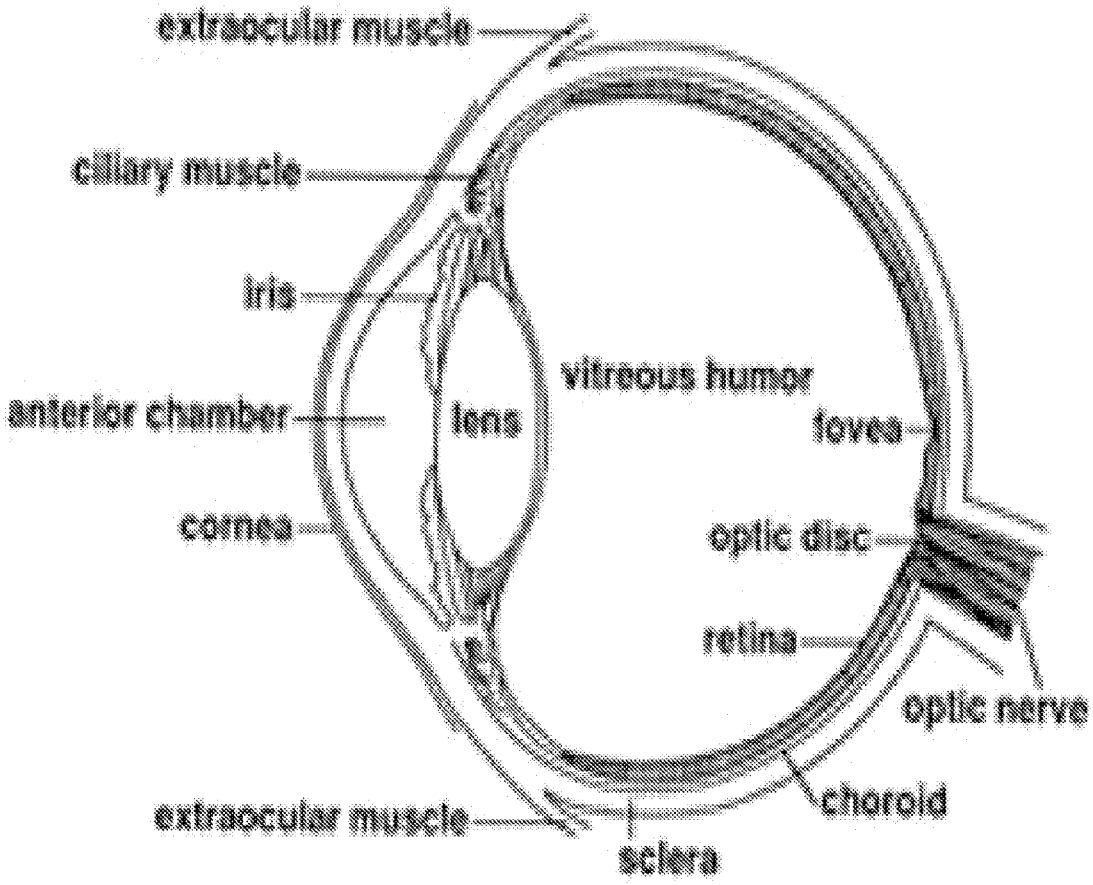

Figure 2.1
Schematic diagram of the eye

FIG. 10

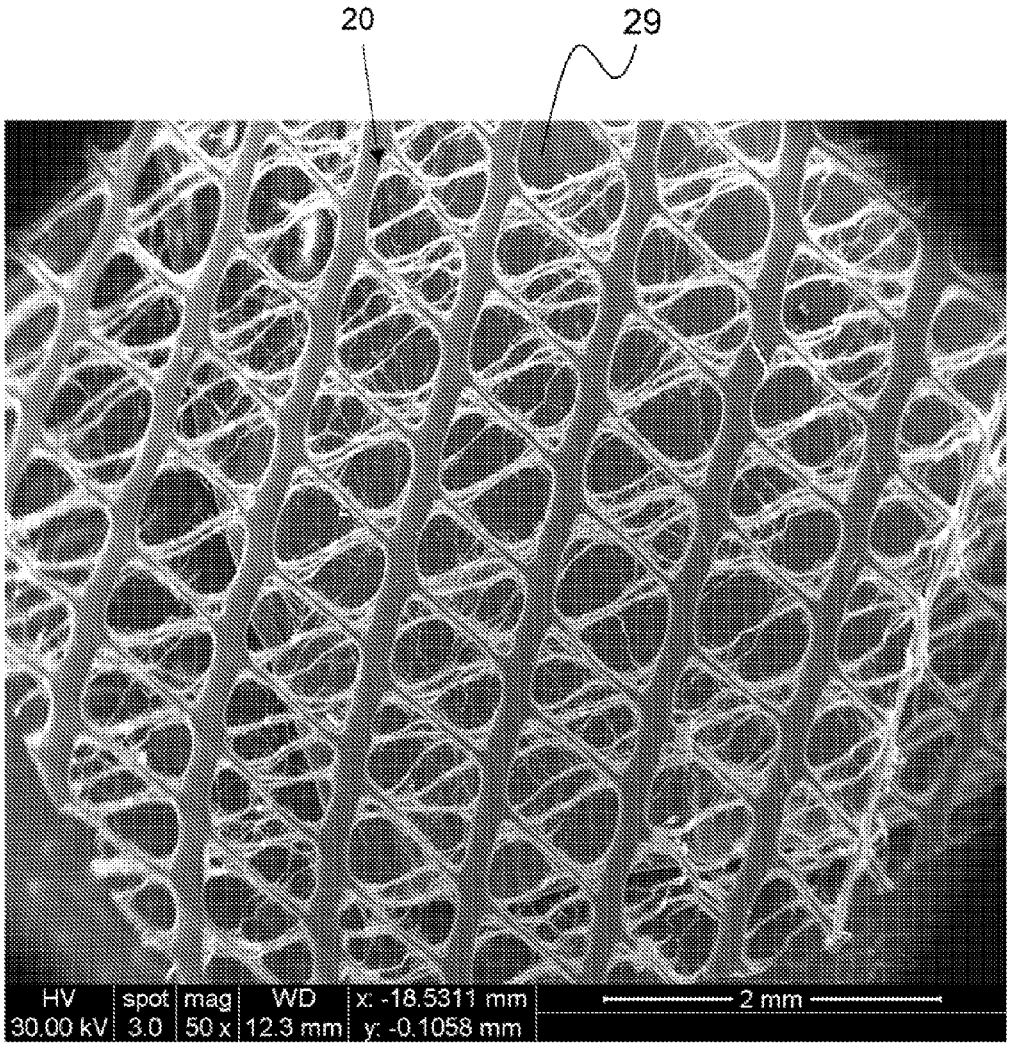
20
29
HV
30.00 kV
spot
3.0
mag
50 x
WD
12.3 mm
x: -18.5311 mm
y: -0.1058 mm
2 mm

FIG. 11

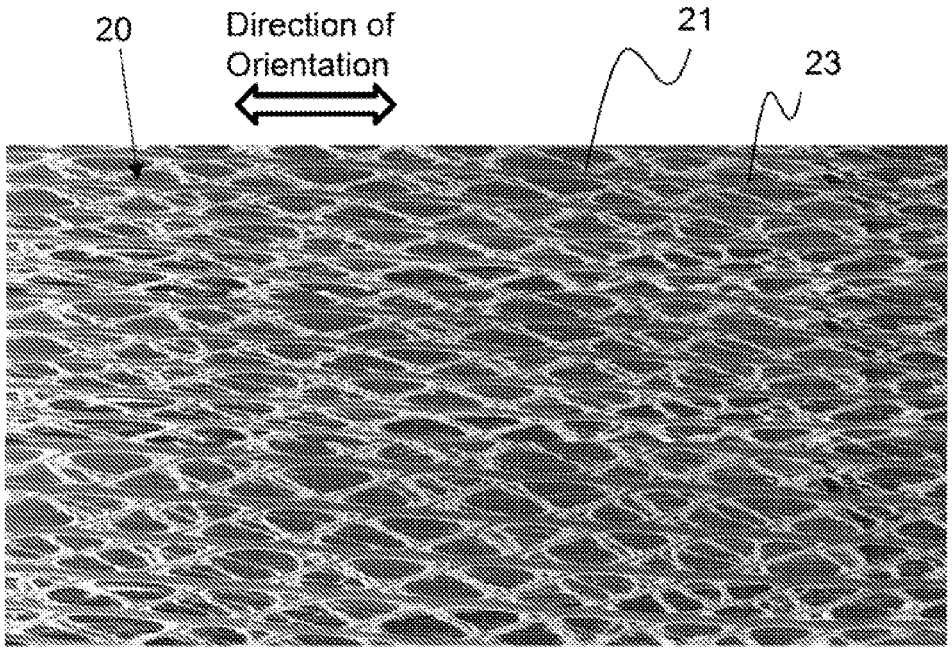

FIG. 12A

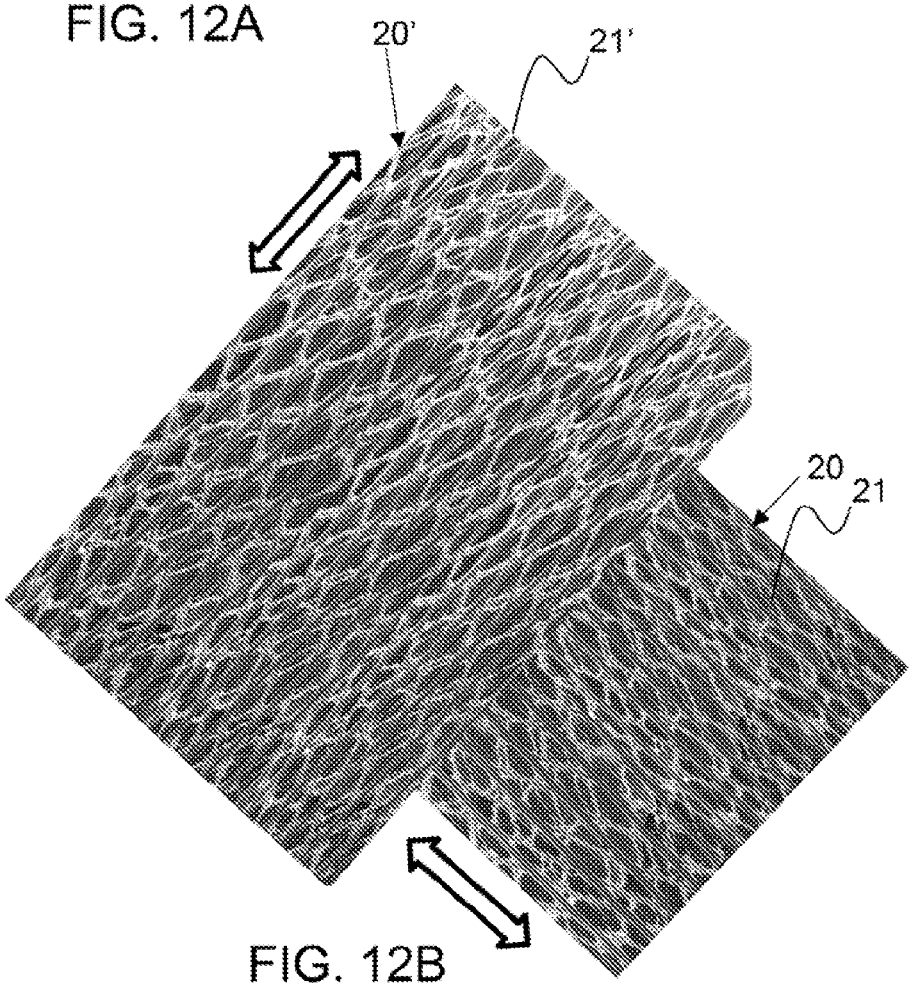

FIG. 12B

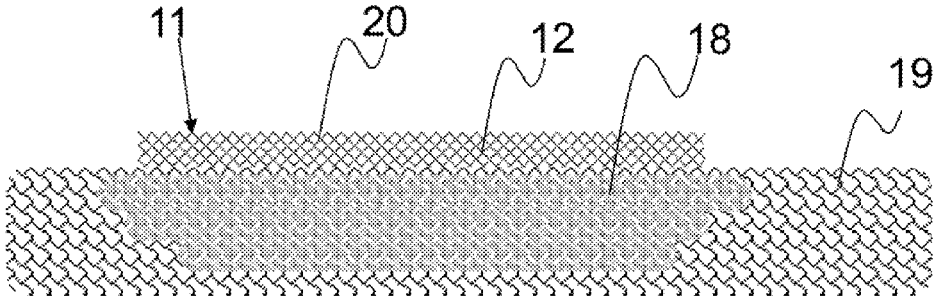

FIG. 13

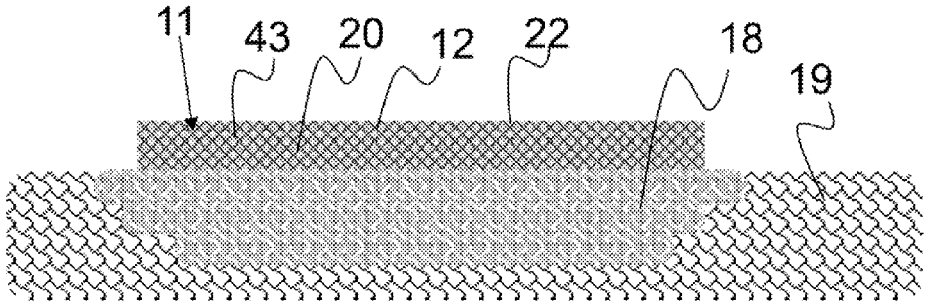

FIG. 14

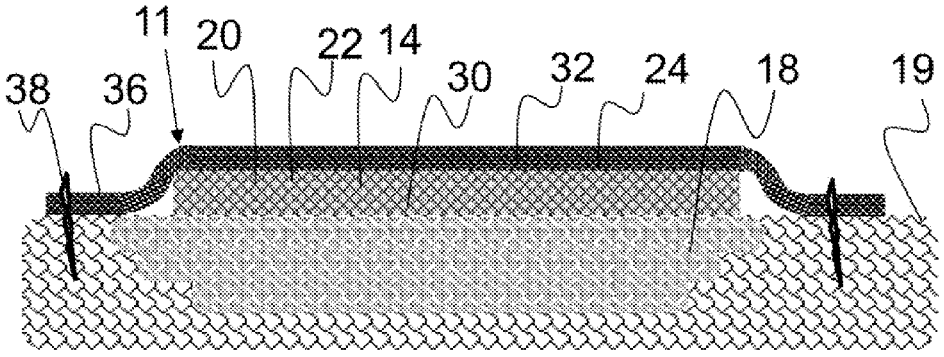

FIG. 15

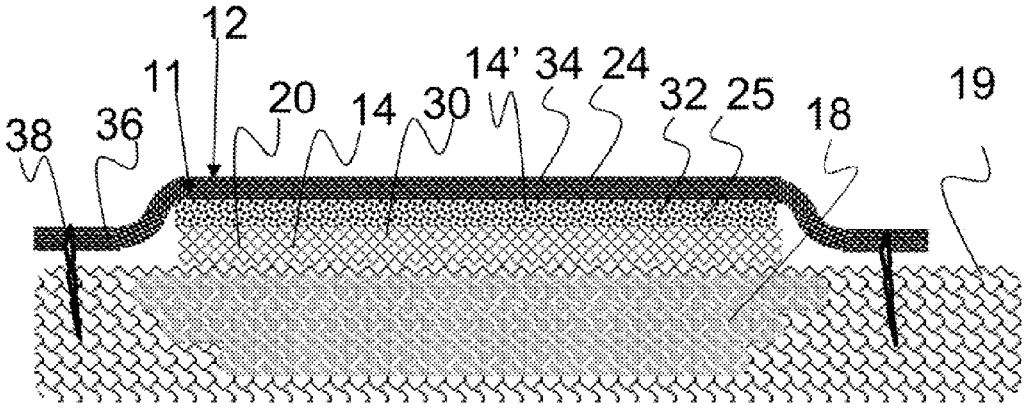

FIG. 16

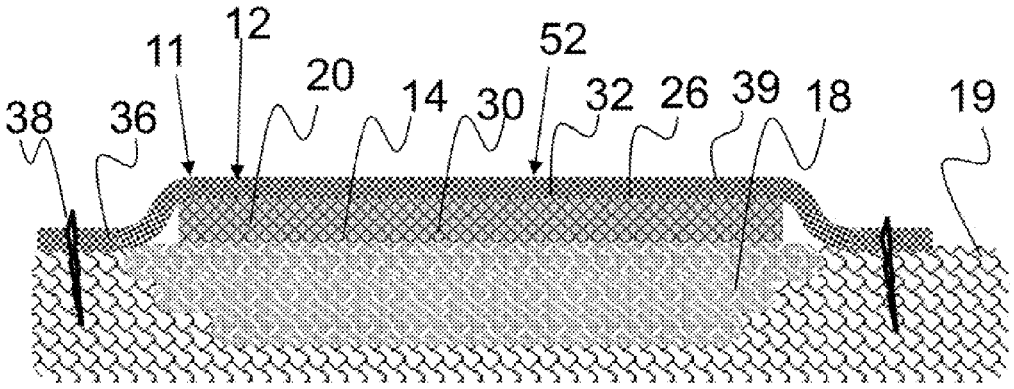

FIG. 17

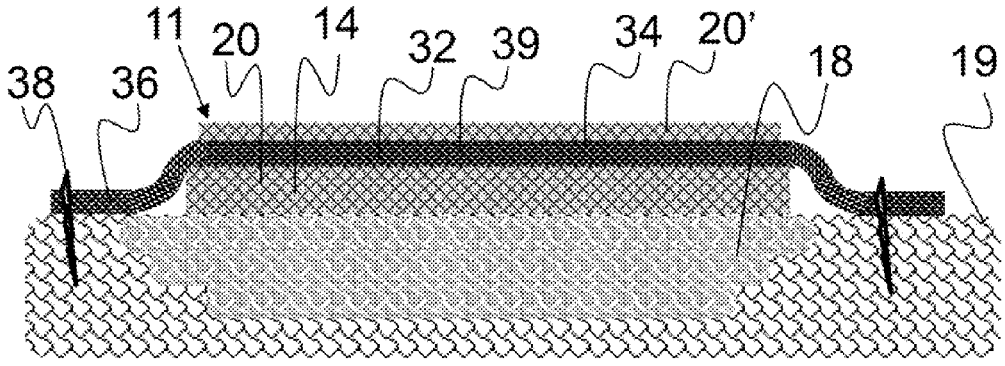

FIG. 18

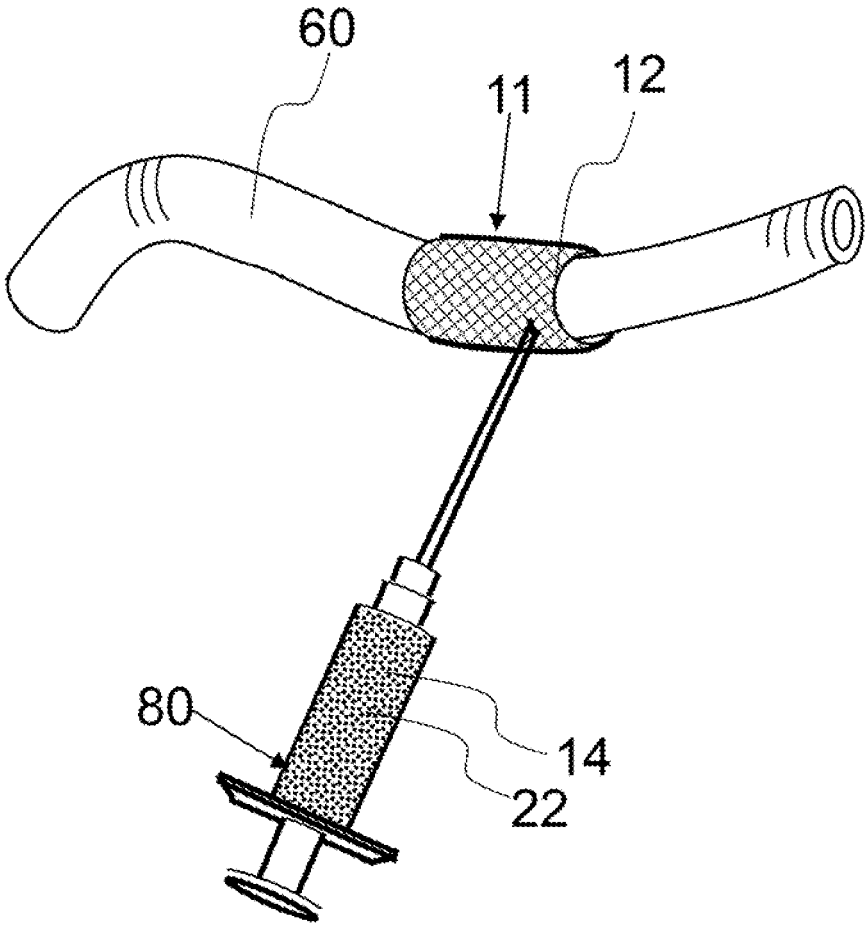

FIG. 19

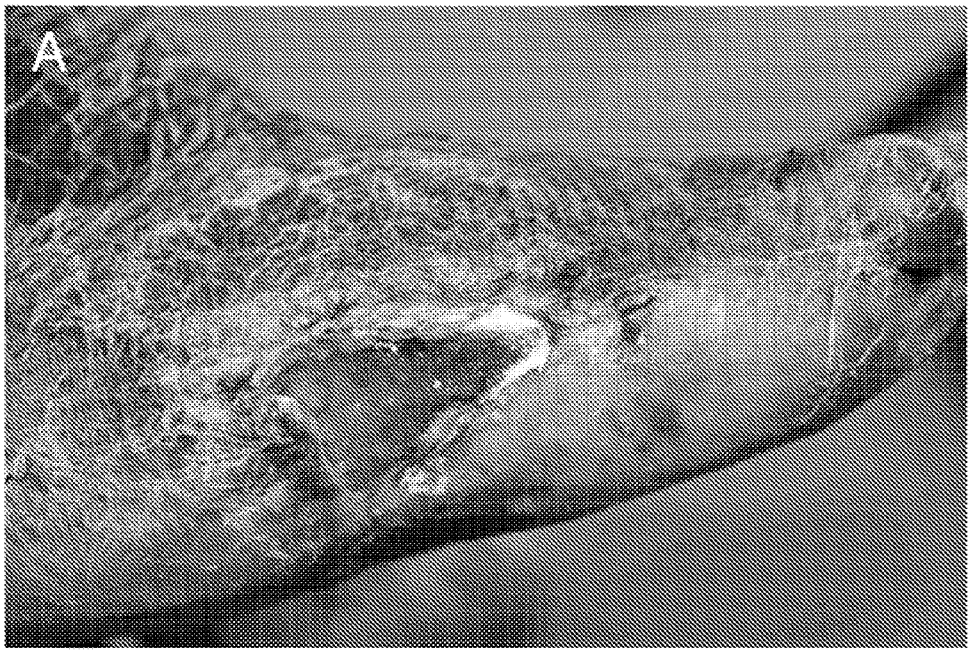

FIG. 20

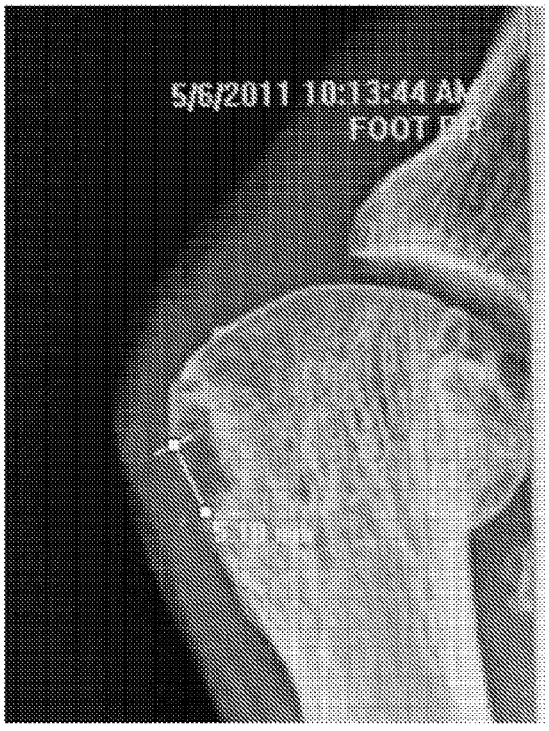

FIG. 21

ACELLULAR AMNION DERIVED THERAPEUTIC COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application No. 62/012,394, filed on Jun. 15, 2014 and entitled Acellular Amnion Derived Therapeutic Compositions; the entirety of which is incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to therapeutic compositions comprising amnion derived acellular materials and methods of use.

2. Background

Amnion derived materials are being used to treat a wide array of conditions such as to promote tissue healing. A widely known practice is to treat a treatment location with amniotic material comprising live amniotic stem cells. This requires special handling, as the amniotic stem cells are fragile and will become inactive if not maintained in a proper condition, including temperature.

Amniotic material that is free of amniotic stem cells or that comprises no live or active amniotic stem cells may be a useful treatment composition and would require less stringent handling.

SUMMARY OF THE INVENTION

The invention is directed to a therapeutic composition comprising amnion derived material that is free of live, or viable cells including amniotic stem cells. An acellular amnion derived material includes at least one of the following: amniotic membrane, amniotic fluid, and/or the following derived from the amniotic membrane and/or amniotic fluid: proteins, extra-cellular proteins such as annexin, fibronection, vitronectin, growth factors, cytokines, collagen and the like. An acellular amnion derived therapeutic composition may comprise amniotic stem cells that are not live or active. In some cases, the cell wall of an amniotic stem cell is ruptured and in other embodiments, essentially all complete and intact amniotic cells are removed from the acellular amnion derived therapeutic composition. In some embodiments, an amnion material, comprising growth factors and/or cytokines, is concentrated in a therapeutic composition, whereby the concentration is higher than in the received donor tissue or fluid. Additional materials including, but not limited to, carriers, diluents or a second therapeutic composition may be included with the amnion derived therapeutic composition. A second composition may comprise live or viable cells, including stem cells. Specific protein markers may be identified and measured to determine the concentration of the amnion derived components with a therapeutic composition.

An acellular amnion derived composition is a material derived from amnion material but contains essentially no live amniotic cells. In an exemplary embodiment, an amnion derived acellular composition comprises no live or active amniotic derived cells. In another embodiment, an acellular amnion derived therapeutic composition comprises essentially no intact amniotic derived cells. In yet another embodiment, an acellular amnion derived therapeutic composition is decellularized and comprises a reduced quantity of cells, such as no more than about five percent, no more than about three percent, no more than about two percent, or no more than about one percent of an original cell concentration. As described herein, during the micronizing of the amniotic membrane, some cells may be fractured, leaving cell fragments and essentially no intact cells. An acellular amnion derived therapeutic composition may comprise live cells that are not derived from amnion however.

An acellular material, as used herein, is defined as a material having essentially no viable cells wherein no more than 1% of the total number of cells in the material are viable. In an exemplary embodiment, an acellular composition contains no viable cells. In an exemplary embodiment, an acellular composition is essentially cell free. Essentially cell free, as used herein, is defined as a composition that contains essentially no cells, wherein the cells have been removed or destroyed through cryo-fracturing, for example.

An acellular amnion derived therapeutic composition may comprise amniotic membrane and/or collagen to provide a scaffold for native cells to grow into. In an exemplary embodiment, an acellular amnion derived therapeutic composition comprises micronized particles of amniotic membrane. In still another embodiment, an acellular amnion derived therapeutic composition comprises a concentrated amniotic fluid. Amniotic stem cells may be present in an acellular amnion derived therapeutic composition, but they are not active and may be ruptured or otherwise physically compromised. Any of the amniotic derived materials described herein may be concentrated by removal of water or other fluids and may have a concentration that is at least about 10% higher, at least about 25% higher, at least about 50% higher, at least about 100% higher, at least about 200% higher than a concentration as received in the donor material.

Amniotic cells including amniotic stem cells may be removed and/or made inactive. Amnion material, including amniotic membrane and amniotic fluid may be decellularized, made essentially acellular as defined herein, through any effective means including, but not limited to, centrifugation, lysis, freezing, filtration, precipitation, flow sorting, sonication and through chemical treatment. Centrifugation may be used to reduce the number of cells within the amniotic material or fluid. Amniotic fluid may be decellularized through centrifugation to reduce the number of cells down to about 750,000 or less intact cells per ml of amniotic fluid, about 500,000 or less intact cells per ml of amniotic fluid, about 450,000 or less intact cells per ml of amniotic fluid, about 300,000 or less intact cells per ml of amniotic fluid, or about 150,000 or less intact cells per ml of amniotic fluid. An as received amniotic fluid from a donor may comprise about 15,000,000 cells per ml before centrifugation and therefore the cell concentration may be reduced to about five percent or less, about three percent or less, about two percent or less, or about one percent or less of the original cell concentration. Cells may be destroyed to produce cell fragments by the addition of chemicals that cause the cell wall of the amniotic stem cells to rupture, thereby making them inactive. The amniotic stem cells may be removed through sonication or filtration, for example. In other embodiments, the amniotic stem cells are made inactive but the cells or cell debris may be left in the amnion derived therapeutic composition.

An acellular amnion derived therapeutic composition may be provided in a form for direct application to a treatment location, such as by topical application, spraying or use of an eye dropper, for example. In other embodiments, an acellular amnion derived therapeutic composition is provided with an applicator such as a sponge, gauze, or a biological applicator, such as an amniotic membrane or composite incorporating amniotic membrane. In still another embodiment, an acellular

amnion derived therapeutic composition may be coated onto an applicator in specific locations to enhance healing, for example.

Any suitable treatment protocol may be used to administer an acellular amnion derived therapeutic composition to a treatment location. In one embodiment, an acellular amnion derived therapeutic composition is applied along with or subsequent to the application of an amniotic composition comprising live amniotic stem cells. For example, a therapeutic dose of live amniotic stem cells may be applied to a treatment location and a separate dose of acellular amnion derived therapeutic composition may be applied to the same treatment location. In addition, subsequent applications of an acellular amnion derived therapeutic composition may be applied to the treatment location to enhance the effectiveness of the treatment.

In another treatment protocol, stem cells derived from a secondary source may be applied to a treatment location and an acellular amnion derived therapeutic composition may also be applied to said treatment location. The secondary source may be from a patient and the stem cells may be derived from adipose tissue or a stromal vascular fraction (SVF), for example. In still another embodiment, live stem cells derived from a secondary source, such as a stromal vascular fraction, may be added to an acellular amnion derived therapeutic composition to create an acellular amnion derived therapeutic composition comprising live non-amnion derived stem cells and an acellular amnion derived component. In this manner, the effectiveness of stem cells derived directly from the patient or a secondary source may be improved by the application of an acellular amnion derived therapeutic composition. The additional growth factors and scaffolding materials applied to the treatment location along with the live stem cells may greatly increase the healing and regenerative effect. It is to be understood that the non-amnion active stem cells may be derived from any suitable location when applied with or combined with an acellular amnion derived therapeutic composition.

In an exemplary embodiment, a SVF comprising live stem cells derived from the tissue of a patient, for example, may be combined with micronized amniotic membrane to form a therapeutic composition for said patient. The SVF may contain any of the following preadipocytes, mesenchymal stem cells (MSC), endothelial progenitor cells, T cells, B cells and mast cells as well as adipose tissue macrophages. In some embodiments, an acellular amnion derived therapeutic composition is doped with progenitor cells and the progenitor cells may be multipotent progenitor cells and/or pluripotent progenitor cells. Progenitor cells may be derived from a patient to be treated, such as from a stromal vascular fraction. Vascular fraction cells and/or progenitor cells may be included with a therapeutic composition to further improve effectiveness. Progenitor cells may be autologous or allogeneic.

An acellular amnion derived therapeutic composition may require cryopreservation as do compositions comprising viable amnion stem cells. In some embodiments, the acellular amnion derived therapeutic composition comprises no live or active stem cells and therefore, there is no requirement to preserve the composition to ensure viability of the stem cells upon thawing. An acellular amnion derived therapeutic composition may be able to be kept at room temperature or refrigerated for long periods of time prior to administering to a treatment location.

In one embodiment an acellular amnion derived therapeutic composition, as described herein, comprises particles of micronized amniotic membrane and/or non-active or destroyed amniotic stem cells. In one embodiment an acellular amnion derived therapeutic composition is a dispersion of micronized amniotic membrane combined with a fluid, such as plasma, saline, amniotic fluid, combinations thereof and the like. In one embodiment, the acellular amnion derived therapeutic composition consists essentially of a mixture of micronized amniotic membrane particles combined with amniotic fluid. In one embodiment the amniotic fluid is a concentrated acellular amniotic fluid, having a concentration of an amnion derived growth factor of at least about 0.1 pg/ml. In one embodiment, the acellular amnion derived therapeutic composition consists essentially of a mixture of micronized amniotic membrane particles, amniotic fluid and saline. An acellular amnion derived therapeutic composition may comprise a concentration of proteins, growth factors, and cytokines that is much higher than the concentration as received in the donor material.

The micronized amniotic membrane may comprise hydrated mammalian amniotic tissue having a percent hydration of at least about 25%, at least about 50%, at least about 75% by weight or any range between the concentrations provided. Amniotic membrane maintained in a hydrated state may provide for more regenerative properties. The particles in the therapeutic composition as described herein may consist essentially of amniotic membrane and be essentially free of chorion. The amnion layer may be removed from the chorion prior to processing. In one embodiment, the amniotic membrane particles consist essentially of epithelium wherein the concentration of the epithelium is about 70% or more, for example. The particles consisting essentially of epithelium may comprise stem cells and tissue that may substantially surround the stem cells. The amniotic membrane particles may be derived from dehydrated and/or decellularized amniotic tissue. An amniotic membrane may be cryo-fractured, such as with a blunt object to minimize shear and damage to the tissue, thereby improving therapeutic effectiveness. Particles of amniotic membrane may have any suitable particle size, average particle size and particle size distribution. For example, the amniotic membrane derived particles, or micronized particles, may have a particle size, or an average particle size of no more than about 100 μm, no more than about 75 μm, no more than about 50 μm, no more than about 25 μm, no more than about 10 μm and any range between and including the average particle sizes provided. The average particle size of the amniotic membrane particles can be determined through any suitable method, including image analysis, whereby a therapeutic composition is dried and imaged using a scanning electron micrograph (SEM). The amniotic membrane derived particles may have an irregular shape and in some embodiments may be planar having a first planar surface and a second planar surface. In some embodiments the amniotic membrane derived particles may be elongated, having a length that is at least three times a cross-length dimension. Cryo-fracturing of amniotic membrane with a blunt object provides particles with less shear and a more irregular shape than conventional cryo-milling, thereby providing a higher surface area and more effective therapeutic effect.

The concentration of particles, such as micronized amniotic membrane, in the therapeutic composition may be provided in any effective amount such as more than about 0.1%, more than about 0.5%, more than about 1%, more than about 10%, more than about 25%, more than about 50%, more than about 75%, or more than about 90% by weight of therapeutic composition and any range between and including the weight percentages listed. Likewise, the mass of particles, such as amniotic membrane particles, may be provided in a therapeu-

tic composition in any effective amount, such as more than about 0.1 m/ml more than about 1 mg/ml, more than about 5 mg/ml, more than about 10 mg/ml, more than about 50 mg/ml, more than about 100 mg/ml, more than about 500 mg/ml, and any range between and including the mass concentrations provided. The particles in the therapeutic composition may comprise collagen, growth factors, stem cells, amniotic stem cells, mesenchymal stem cells, progenitor cells, red blood cells, white blood cells, proteins, fibroblasts, paratenacytes, keratinocytes and the like.

Additional fluids and agents may be added to the acellular amnion derived therapeutic composition including, but not limited to, Plasma Lyte-A, from Baxter Inc., saline and the like. An acellular amnion derived therapeutic composition, as described herein, may comprise anti-inflammatory nano-particles and/or statins, and HMG-CoA reductase inhibitors to reduce inflation at a treatment location.

An acellular amnion derived therapeutic composition may comprise proteins, growth factors and cytokins derived from the amnion. Amnion derived protein may be identified in an acellular amnion derived therapeutic composition by a protein marker including, but not limited to, basic fibroblast growth factors (bFGF), bone morphogenetic protein 2 (bmp-2), bone morphogenic protein 4 (bmp4), bone morphogenetic protein 7 (bmp-7), bone morphogenic protein 9 (bmp-9), epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor AA (PDGF-AA), platelet growth factor BB (PDGF-BB), platelet growth factor AB (PDGF-AB), transforming growth factor beta one (TGF-b1), and vascular endothelial growth factor (VEGF). Flow cytometry may be used to identify proteins markers such as, CD44, CD105, CD73, CD90, CD29, CD166, CD58 and other proteins found in amnion material. It is to be understood that any number of protein markers common to amniotic material may be identified in a composition to determine if the composition is amnion derived. Any other material derived from amnion material including the membrane and fluid may be included in an acellular amnion derived therapeutic composition, as described herein.

An acellular amnion derived therapeutic composition may be decellularized, made acellular, through any suitable means including, but not limited to, sterilization, lyophilizing, freezing, centrifuging, radiation exposure and the like. In some embodiments, a therapeutic composition is acellular through a process of destroying or making inactive any live cells, such as amniotic stem cells. In another embodiment, essentially all cells, including amniotic stem cells, are removed from the therapeutic composition through filtration and/or centrifugation wherein no more than about five percent, no more than about three percent, no more than about two percent, or no more than about one percent of an original quantity of intact cells remains in a therapeutic composition, as described herein. In yet another embodiment, a therapeutic composition is acellular, comprising a plurality of dead cells, such as amniotic stem cells. Dead and/or destroyed cells may release proteins and growth factors into the therapeutic composition. An acellular therapeutic composition may comprise particles of amniotic membrane, such a cyrofractured or morselized amniotic membrane, as described herein. In another embodiment, an acellular amnion derived therapeutic composition consists of a fluid component that is essentially free of cells. For example, amniotic fluid may be centrifuged to substantially remove all the amniotic stem cells, including dead cells. In one embodiment, an acellular amnion derived therapeutic composition is sterilized and then stored at ambient temperature, or refrigerated to a temperature of greater than 0° C. or greater than −15° C. prior to use.

Any of the acellular amnion derived therapeutic compositions described herein may be an injectable solution that will pass through a 30 gauge needle or larger diameter needle. An acellular amnion derived therapeutic composition may be provided to a patient transcatheter. In other embodiments, an acellular amnion derived therapeutic composition is provided in a thicker composition, such as a paste that may be applied topically. The viscosity of the injectable acellular amnion derived therapeutic composition may be no more than about 1 mPa sec. no more than about 500 mPa sec, no more than about 1000 mPa sec. no more than 20,000 mPa sec, no more than 50,000 mPa sec and any range between and including the viscosity values provided.

In other embodiments, an acellular amnion derived therapeutic composition may be provided for topical applications and the viscosity may be more than about 20 Pa sec, more than about 50 Pa sec, more than about 100 Pa sec, more than about 250 Pa sec and any range between and including the viscosity values provided.

The acellular amnion derived therapeutic composition described herein may be cryopreserved, whereby the temperature of the therapeutic composition is lowered to a temperature of no more than −70° C., and preferably lower than about −80° C. The rate of cooling may be controlled to reduce damage and maintain viability of the cells upon thawing.

An acellular amnion derived therapeutic composition, as described herein, may comprise an amniotic membrane to create an acellular therapeutic composite. A therapeutic composite comprising an amniotic membrane may be used in any suitable treatment method of use, as described herein. An amniotic membrane may be provided in a multilayered configuration or combined with any other suitable matrix component for a desired application. For example, an acellular therapeutic composite, as described herein, may comprise an amniotic membrane layer and a cover layer. A cover layer may be used to reduce the loss or wash-out of a fluid component from the acellular therapeutic composite. In another embodiment, the acellular therapeutic composite comprises an amniotic membrane and a support layer, such as a polymer matrix material including, but not limited to, a bioresorbable or fluoropolymer membrane. A support layer may have a tensile break strength that is much greater, such as two times or more, than that of an amniotic membrane layer in a matrix component. In still another embodiment, a acellular amnion derived therapeutic composite comprises one or more layers of amniotic membrane that are tensilized, whereby an amniotic membrane has been stretched in one or more directions to increase strength and/or area of the membrane. An amniotic membrane may be cross-linked, and a cross-linked amniotic membrane may be combined with a non-cross-linked amniotic membrane. Any suitable method as known in the art of cross-linking an amniotic membrane may be used including, but limited to, chemical treatment with glutaraldehyde, radiation and the like.

In another embodiment, a fluid component of an acellular amnion derived composite comprises amniotic membrane that has been micronized and dispersed in a fluid. In one embodiment, a fluid component is a dispersion of micronized amniotic membrane combined with a fluid, such as plasma, saline, amniotic fluid, combinations thereof and the like. In an exemplary embodiment, the fluid component and amniotic membrane are from a single donor. A fluid component, as described herein, may comprise anti-inflammatory nano-particles and/or statins, or HMG-CoA reductase inhibitors to reduce inflation at a treatment location.

An acellular amnion derived therapeutic composite, as described herein, may be provided with the fluid component

imbibed into, coated onto or otherwise applied to a matrix component. For example, an acellular amnion derived therapeutic composite comprising an amniotic membrane may be provided with a fluid component comprising micronized amniotic membrane particles dispersed in concentrated acellular amniotic fluid component. In an exemplary embodiment, the amniotic membrane and a fluid component are all from a single donor. In another exemplary embodiment, a therapeutic composite comprises an amniotic membrane layer configured for direct application to a treatment location, a cover layer of a bioresorbable material and a fluid component. A portion of a bioresorbable material or other matrix layer of the therapeutic composite may be porous to enable a portion of the fluid component to be retained therein. Any suitable number and type of matrix or support layers may be configured in a therapeutic composite, as described herein. In one embodiment, a fluid component may be vacuum imbibed into a matrix component; whereby a matrix component is submerged in a fluid component and vacuum is applied to remove air from the matrix component. This removal of air allows the fluid component to more substantially fill the voids and porosity of the matrix component.

A support layer may comprise any suitable type of material including, but not limited to, a bioresorbable material, a non-bioresorbable polymer material, such a polyether ether ketone (PEEK), or polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), perfluoroalkoxy (PFA) and the like, or a metallic component, such as stainless steel, titanium, gold and the like. A support layer may be porous and/or permeable. A support layer may be a membrane having a microstructure of pores, or a film, net, screen, woven and the like. A support layer may be substantially non-permeable to fluid and may be hydrophobic or oleophobic on at least one side. In an exemplary embodiment, a support layer is expanded PTFE. In an exemplary embodiment, a support layer is a sheet of material having a first substantially planar surface, a second substantially planar surface and a thickness.

Any of the acellular amnion derived therapeutic composition described herein may be used for a wide variety of treatment applications including, but not limited to, any organ, respiratory system, circulatory system, digestive system and the like. A therapeutic composition, as described herein, may be provided to any suitable treatment location of the body to induce an immunomodulatory and/or anti-inflammatory response. In another application, a therapeutic composition is introduced into a treatment location to reduce scaring and to promote healing, whereby the therapeutic composition aids in regeneration of new tissue. A therapeutic composition may be injected directly into an affected area or introduced intravenously.

An effective dose of an acellular therapeutic composition may be provided in one treatment or in several doses over a period of time. The specific treatment and dosing regime will depend on the type and severity of the condition to be treated.

In one embodiment, an acellular amnion derived therapeutic composition is injected into a specific treatment location through the use of a catheter, such as a steerable catheter and an injection implement configured on the introductory end of the catheter. For example, a catheter having an injection implement may be introduced to an artery, inserted to position the injection implement in proximity of the treatment location, whereby a dose of therapeutic composition is administered into the treatment location.

An acellular amnion derived therapeutic composition, as described herein, may be used in conjunction with any suitable matrix component including bioresorbable materials, synthetic polymer material and membranes and the like. The therapeutic composition may be imbibed into, coated onto or otherwise combined with a matrix component for treatment. In an exemplary embodiment, a therapeutic composition is coated into a porous bioresorbable material and placed onto a treatment location. In an exemplary embodiment, a therapeutic composition is combined with a patch that contains a plurality of nano-needles, and the patch is applied to a treatment area.

An acellular amnion derived therapeutic composition may be a cosmetic composition and comprise one or more cosmetic components, as defined herein. An acellular amnion derived therapeutic composition may be a cosmetic composition that is configured for topical application to the skin of a subject to reduce wrinkles, discolorations, improve appearance and the like. Cosmetic composition, as used herein, is defined as any substance or preparation intended to be paced in contact with the various external parts of the human body for the purpose to clean, perfume, change the appearance, protect, keep in good condition, or correct body odors. A cosmetic composition may comprises any suitable combination of cosmetic components including, but not limited to, water, alcohols such as polyhydric, ethylene glycol, propylene glycol, trimethylene glycol, butylene glycol, isoprene glycol and sorbitol, hydrocarbon polymers, silicone polymers, silicone emollient, silicone oligomer, natural oils derived from plants or animals, such as fruit or vegetable derived oils, mineral oil, wax, borax, acids including polylactic acids and surfactants.

An acellular amnion derived therapeutic composition may be made from amnion tissue and/or fluid from any suitable mammalian donor, including humans, horses, pigs, and the like. In addition, an acellular amnion derived therapeutic composition may be used to treat a treatment location of any suitable mammalian patient, including a human or horse for example.

The summary of the invention is provided as a general introduction to some of the embodiments of the invention, and is not intended to be limiting. Additional example embodiments including variations and alternative configurations of the invention are provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate embodiments of the invention, and together with the description serve to explain the principles of the invention.

DETAILED DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

FIG. **1**A shows a cross-sectional diagram of amniotic membrane surrounding a fetus in utero.

FIG. **1**B shows a cross-sectional diagram of the layers of the amnion and chorion.

FIG. **2**A shows a transmission electron micrograph (TEM) of the epithelium layer of the amniotic membrane having a single layer of amniotic stem cells. The TEM was taken at 2500× magnification.

FIG. **2**B shows a TEM of the epithelium layer of the amniotic membrane having a single layer of amniotic stem cells. The TEM was taken at 8200× magnification.

FIG. **3**A is a scanning electron micrograph (SEM) of an amniotic membrane having amniotic stem cells.

FIG. **3**B is a SEM of cryo-fractured amniotic membrane particles.

FIG. **4** shows a diagram of a process to produce an acellular amnion derived therapeutic composition comprising micronized amniotic membrane particles.

FIG. **5** shows a diagram of a process to produce an acellular amnion derived therapeutic composition comprising a concentrated amniotic fluid.

FIG. **6** shows a diagram of the anatomy and various organs within the body.

FIG. **7** shows a diagram of the circulatory system.

FIG. **8** shows an exemplary an acellular amnion derived therapeutic composition being drawn from an enclosure by a needle.

FIG. **9** shows a knee joint and a syringe injecting an acellular amnion derived therapeutic composition into the knee joint.

FIG. **10** shows a cross-sectional diagram of an eye.

FIG. **11** is a scanning electron micrograph (SEM) of an amniotic membrane having pores between the amniotic membrane tissue.

FIG. **12**A is a representation of an exemplary tensilized amniotic membrane.

FIG. **12**B is a representation of two exemplary tensilized amniotic membranes being layered together.

FIG. **13** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite comprising an amniotic membrane configured over a treatment location.

FIG. **14** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite comprising an amniotic membrane and fluid component configured over a treatment location.

FIG. **15** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite configured over a treatment location wherein the therapeutic composite comprises an amniotic membrane imbibed with a fluid component and a cover layer configured there over.

FIG. **16** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite configured over a treatment location wherein the therapeutic composite comprises a first matrix layer of amniotic membrane, a second matrix layer of a fluid component reservoir, and a third matrix layer that is a cover layer.

FIG. **17** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite configured over a treatment location wherein the therapeutic composite comprises a first matrix layer of amniotic membrane imbibed with fluid component and a second matrix layer that is a support layer comprising bioresorbable material.

FIG. **18** shows a cross-sectional representation of an exemplary acellular amnion derived therapeutic composite configured over a treatment location wherein the therapeutic composite comprises a first matrix layer of amniotic membrane imbibed with fluid component, a second matrix layer that is a support layer and a third matrix layer that comprises amniotic membrane.

FIG. **19** shows an exemplary therapeutic composite configured around a ureter and a fluid component being injected therein.

FIG. **20** shows a picture of a wound on a diabetic person's foot prior to treatment.

FIG. **21** shows an x-ray of an osteochondral defect in an ankle, prior to treatment.

Corresponding reference characters indicate corresponding parts throughout the several views of the figures. The figures represent an illustration of some of the embodiments of the present invention and are not to be construed as limiting the scope of the invention in any manner. Further, the figures are not necessarily to scale, some features may be exaggerated to show details of particular components. Therefore, specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present invention.

As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having" or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. Also, use of "a" or "an" are employed to describe elements and components described herein. This is done merely for convenience and to give a general sense of the scope of the invention. This description should be read to include one or at least one and the singular also includes the plural unless it is obvious that it is meant otherwise.

Certain exemplary embodiments of the present invention are described herein and illustrated in the accompanying figures. The embodiments described are only for purposes of illustrating the present invention and should not be interpreted as limiting the scope of the invention. Other embodiments of the invention, and certain modifications, combinations and improvements of the described embodiments, will occur to those skilled in the art and all such alternate embodiments, combinations, modifications, improvements are within the scope of the present invention.

As shown in FIG. **1**A the amniotic membrane surrounds a fetus in utero. As shown in FIG. **1**B, the amniotic membrane comprises an amnion portion and a chorion portion. As described herein, the amnion portion may be separated from the chorion. In an exemplary embodiment, the epithelium, or inner most layer of the amniotic membrane, is removed and used to produce particles for the acellular amnion derived therapeutic composition, as described herein. The particles may consist essentially of the epithelium, consist essentially of the epithelium and base membrane, consist essentially of the epithelium, base membrane and compact layer, or consist essentially of epithelium, base membrane, compact layer, and fibroblast layer.

As shown in FIGS. **2**A and **2**B, the epithelium layer of the amniotic membrane **20** has a single layer of amniotic stem cells. The tissue around the amniotic stem cells may protect and enhance the viability of these stem cells when the epithelium is cryo-fractured to produce particles for the therapeutic composition.

As shown in FIG. **3**A, an amniotic membrane **20** comprises a plurality of amniotic stem cells **32**.

As shown in FIG. **38**, particles of cryo-fractured amniotic membrane particles are on the order of 0.2 to 0.5 μm in size. The average particle size shown is less than 2 μm. There are no particles shown that are larger than 2 μm and substantially all of the particles are less than 1 μm in size. The SEM shows that the micronized amniotic membrane particles are irregularly shaped. As shown, some of the particles have a planar surface.

As shown in FIG. **4**, a process to produce an acellular amnion derived therapeutic composition, as described herein, comprises the steps of cryo-fracturing amniotic membrane fragments to produce micronized amniotic membrane particles. As described, the amniotic membrane fragments may be cryo-fractured with a blunt object, such as a bar, that reduces shear and damage to the particles. In a preferred embodiment, the fragments are cryo-fractured with an object having substantially no sharp edges. The micronized particles

are combined with any suitable carrier fluid to produce an acellular amnion derived therapeutic composition. In an exemplary embodiment, the micronized particles are dispersed in a fluid comprising amniotic fluid. The cells in the amniotic membrane may be destroyed prior to or after the process shown in FIG. **4**, or between any of the steps.

As shown in FIG. **5**, a process to produce an acellular amnion derived therapeutic composition, as described herein, comprises the steps of concentrating an amniotic fluid component comprising Plasma Lyte-A, available from Baxter Inc. An amniotic fluid may be processed in any suitable way to concentrate the amniotic fluid and components therein. For example, an amniotic fluid may be heated or allowed to evaporate, with or without vacuum, to concentrate the amniotic fluid. The cells in the amniotic fluid may be destroyed prior to or after the process shown in FIG. **5**, or between any of the steps.

FIG. **6** shows a diagram of the anatomy and various organs within the body that may be treated with an acellular amnion derived therapeutic composition as described herein. An acellular amnion derived therapeutic composition, as described herein, may be introduced into any anatomy shown in FIG. **6** by direct injection, topical application, or transcatheter.

FIG. **7** shows a diagram of the circulatory system where an acellular amnion derived therapeutic composition may be introduced into the body through injection or transcatheter.

FIG. **8** shows an exemplary acellular amnion derived therapeutic composition **10** being drawn from an enclosure **70** by a syringe **80**. The acellular amnion derived therapeutic composition comprises micronized particles **22** of amniotic membrane **20**. The needle may be any suitable size, however in a preferred embodiment the needle is no larger than a 20 gauge needle.

As shown in FIG. **9**, a syringe **80** is injecting an acellular amnion derived therapeutic composition **10** comprising micronized particles of amniotic membrane **22** dispersed in a fluid component **14** into the knee joint.

FIG. **10** shows a cross-sectional diagram of an eye and some of the treatment locations for an acellular amnion derived therapeutic composition, as described herein. For example, an acellular amnion derived therapeutic composition, as described herein, may be applied topically and/or injected into the iris, anterior chamber, lens, vitreous humor, cilliary muscle, comea, extraocular muscle, sciera, choroid, retina and the like.

As shown in FIG. **11** an amniotic membrane **20** comprises pores **29** between the amniotic membrane tissue. This porosity may be imbibed with an acellular amnion derived therapeutic composition. In addition, an amniotic membrane may be stretched in one or more directions to tensilize the tissue. A tensilized amniotic membrane may have a higher matrix tensile strength than an original un-tensilized amniotic membrane. In addition, a plurality of layers of amniotic membrane may be utilized to build strength in one or more directions.

As shown in FIG. **12**A, an amniotic membrane **20** has been stretched in one direction to form an elongated and more aligned amniotic tissue orientation. As shown in FIG. **12**A, oriented tissue **23** is aligned horizontally and connecting tissue interconnects the oriented tissue. A tensilized amniotic membrane **21** may be stronger by unit weight in the oriented direction and may have a much higher elongation to break in the cross-oriented direction than a precursor amniotic membrane, before tensilizing. The tensilized amniotic membrane **21** may be stretched as much as 120%, 150%, 175%, or 200% of the original membrane length. The amniotic membrane may neck or narrow in the opposing direction of stretch. A stretched or tensilized amniotic membrane may be stretched over a long period of time to minimize tissue fracture. For example, an amniotic membrane may have a low load applied and may be stretched over a period of 10 minutes or more, 30 minutes or more, 1 hour or more, 6 hours or more, 1 day or more, 2 days more and any range between and including the durations provided. In addition, an amniotic membrane may be stretched while being hydrated and/or submerged in amniotic fluid or a plasticizing fluid. An amniotic membrane may be cross-linked after being stretched. The load applied to tensilize an amniotic membrane may be a portion of the maximum tensile load required to fracture the amniotic membrane at a rate of 10 mm/second for a 25.4 mm by 15.24 cm sample having a 50.8 mm gap. For example, a tensilizing load applied may be no more than about 80%, no more than about 60%, no more than about 50%, or no more than about 25% of the maximum tensile load.

As shown in FIG. **12**B, a first tensilized amniotic membrane **20** is configured at a 90 degree offset from a second amniotic membrane **20'**. This orientation of layering may provide for a much stronger therapeutic composite. In an alternative embodiment, a plurality of layers of tensilized amniotic membrane may be aligned with the oriented tissue of a first layer being aligned with the oriented tissue of a second layer. A matrix component or a therapeutic composite, as described herein, may consist essentially of tensilized amniotic membrane.

As shown in FIG. **13**, an exemplary acellular amnion derived therapeutic composite **11** comprises an amniotic membrane **20** configured over a treatment location **18** in the tissue **19** of a subject. The matrix component **12** in this embodiment consists essentially of amniotic membrane **20**.

As shown in FIG. **14**, an exemplary acellular amnion derived therapeutic composite **11** comprises an amniotic membrane **20** and fluid component **14** configured over a treatment location **18**. The fluid component **14** comprises micronized amniotic membrane particles **22** and amniotic fluid **43**. Any suitable fluid carrier may be used to disperse the micronized amniotic membrane particles and/or amniotic fluid.

As shown in FIG. **15**, an exemplary acellular amnion derived therapeutic composite **11** is configured over a treatment location **18** wherein the therapeutic composite comprises an acellular amniotic membrane **20** imbibed with a fluid component **14** and a cover layer **24** is configured there over. The matrix component **12** comprises a first matrix layer **30** and a second matrix layer **32**. The second matrix layer is configured over said first matrix layer and comprises an overhang portion **36** that extends outside of the first matrix layer. The second matrix layer is attached to the tissue **19** by an attachment component **38**, such as a staple, glue and/or sutures, for example. A matrix component, or a layer of a matrix component, may be configured to extend beyond a treatment location, whereby an outer area of the matrix component can be affixed to tissue. A cover layer may fully cover a first or under layer of matrix component or may only cover a portion of a layer thereunder. A cover layer may be a net or mesh or strands that extend across and over an under-layer, for example.

As shown in FIG. **16**, an exemplary acellular amnion derived therapeutic composite **11** is configured over a treatment location **18**. The acellular therapeutic composite **11** comprises a matrix component **12** comprising a first matrix layer **30** of amniotic membrane **20**, a second matrix layer **32** of a fluid reservoir layer **25**, and a third matrix layer **34** that is a cover layer **24**. The fluid reservoir layer comprises a matrix having porosity containing a fluid component **14'**, as described herein. As shown, a first fluid component **14** is

configured within the first matrix layer **30**. It is to be noted that different compositions of a first and second fluid component may be configured in a matrix component **12**.

As shown in FIG. **17**, an acellular amnion derived therapeutic composite **11** is configured over a treatment location **18** wherein the matrix component **12** comprises a first matrix layer **30** of amniotic membrane **20** imbibed with fluid component **14** and a second matrix layer **32** that is a support layer **39** comprising bioresorbable material **26**. The support layer may be substantially impermeable to the fluid component configured in the first matrix component that is proximate a treatment location. In addition, an outer surface **52** of a matrix component **12**, or the surface facing away a treatment location, may be hydrophobic to reduce fluid ingress into the therapeutic composite. Bodily fluid ingress into a therapeutic composite may dilute a fluid component comprised therein.

As shown in FIG. **18**, an exemplary acellular amnion derived therapeutic composite **11** is configured over a treatment location **18** wherein the matrix component **12** comprises a first matrix layer **30** of amniotic membrane **20** imbibed with fluid component **14**, a second matrix layer **32** that is a support layer **39** and a third matrix layer **34** that comprises amniotic membrane **20**. A support layer is configured between amniotic membranes in this embodiment. As described herein, a matrix component may be provided with multiple layers attached and ready for orientation on a treatment location, or a plurality of matrix components may be applied, one after another, during the treatment procedure.

As shown in FIG. **19**, an exemplary acellular amnion derived therapeutic composite **11** is configured around a ureter and a fluid component **14** is being injected therein. This type of procedure may reduce and/or eliminate strictures. A matrix component may be a sheet of material having a substantially planar top and bottom surface and substantially uniform thickness therebetween. A sheet of matrix composite may be supple and may be configured around a cylindrical treatment location, such as a portion of the urinary or digestive system. In another embodiment, a matrix component sheet is applied externally over a treatment location in a patient's dermal tissue. It is to be understood that a composition comprising viable cells may be injected into or otherwise placed into contact with an acellular amnion derived therapeutic composite, as described herein. For example, the syringe **80** shown in FIG. **19** may comprise live viable stem cells that are injected into an exemplary acellular amnion derived therapeutic composite **11**. The stem cells may be any suitable type of stem cells.

As shown in FIG. **20**, a wound on a diabetic person's foot has a length of approximately 11 mm and width of approximately 7 mm. An acellular amnion derived therapeutic composite of amniotic membrane may be placed over the wound and a fluid component comprising micronized amniotic membrane and a concentrated amniotic fluid may be applied topically. Stem cells derived from the patient's stromal vascular fraction may be applied to the treatment location as well.

As shown in FIG. **21**, a patient has an osteochondral defect in an ankle, with some bone degradation. An acellular amnion derived therapeutic composite may be applied over the defect and an acellular amnion derived therapeutic fluid component may then be applied to the treatment site.

DEFINITIONS

An acellular amnion derived composition is a material derived from amnion material but contains essentially no live amniotic cells. In an exemplary embodiment, an amnion derived acellular composition comprises no live or active amniotic derived cells. In yet another embodiment, an acellular amnion derived therapeutic composition comprises essentially no intact amniotic derived cells. As described herein, during the micronizing of the amniotic membrane, cells may be fractured leaving cell fragments and essentially no intact cells.

An acellular material, as used herein, is defined as a material having essentially no viable cells wherein no more than 1% of the total number of cells in the material are viable. In an exemplary embodiment, an acellular composition contains no viable cells. In an exemplary embodiment, an acellular composition is essentially cell free. Essentially cell free, as used herein, is defined as a composition that contains essentially no intact cells, or no more than five percent, no more than three percent, no more than two percent, or no more than one percent of an original intact cell concentration, or no more than about 750,000 intact cells per ml of material, no more than about 450,000 intact cells per ml of material, no more than about 300,000 intact cells per ml of material, or no more than about 150,000 intact cells per ml of material. An essentially cell free material may contain cell fragments that have been destroyed through cryo-fracturing, for example.

Micronized amniotic membrane particles, as used herein, is defined as particles derived from amniotic membrane that have an average particle size of no more than about 100 um and may have an average particle size of no more than about 75 um, no more than about 50 um, no more than about 25 um, no more than about 10 um and any range between and including the average particle sizes provided. Particle size may be measured by analysis of scanning electron micrographs. Micronized amniotic membrane particles may be formed through any suitable method including, but not limited to, cryogenic fracturing, application of heat and pressure, sonication and/or enzyme digestion.

Amniotic fluid may be decellularized to remove a portion of the cells through centrifugation, for example. A decellularized amniotic fluid may be an essentially cell free amniotic fluid obtained through centrifugation, filtration, or other process to remove essentially all of the cells and/or cell debris and may contain essentially no intact cells, or no more than about five percent, no more than three percent, no more than two percent, or no more than one percent of an original intact cell concentration, or no more than about 750.000 intact cells per ml of material, no more than about 450,000 intact cells per ml of material, no more than about 300,000 intact cells per ml of material or no more than about 150,000 intact cells per ml of material.

An intact cell, as used herein, is a cell that is viable or non-viable and retains an original shape and has not been ruptured or split into two or more pieces.

It will be apparent to those skilled in the art that various modifications, combinations and variations can be made in the present invention without departing from the spirit or scope of the invention. Specific embodiments, features and elements described herein may be modified, and/or combined in any suitable manner. Thus, it is intended that the present invention cover the modifications, combinations and variations of this invention provided they come within the scope of the appended claims and their equivalents.

What is claimed is:

1. A therapeutic composition comprising:

a) acellular amniotic membrane particles;

b) a carrier fluid;

wherein the acellular amniotic membrane particles consist essentially of micronized amniotic membrane;

wherein the carrier comprises an acellular amniotic fluid; and

wherein the therapeutic composition is essentially free of any viable amniotic membrane cells or viable amniotic fluid cells.

2. The therapeutic composition of claim **1**, wherein the acellular amniotic fluid is essentially cell free containing no more than 500,000 intact cells per ml of therapeutic composition.

3. The therapeutic composition of claim **1**, wherein the amniotic fluid is a concentrated acellular amniotic fluid, having a concentration of an amnion derived growth factor of at least about 0.1 pg/ml.

4. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles consist essentially of amnion and are essentially free of chorion.

5. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles are decellularized.

6. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles have an average particle size of no more than about 100 μm.

7. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles are irregularly shaped.

8. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles are planar in shape, having a first planar surface and a second planar surface.

9. The therapeutic composition of claim **1**, wherein the micronized amniotic membrane particles are elongated, having a length that is at least three times a cross-length dimension.

10. The therapeutic composition of claim **1**, having a concentration of acellular amniotic membrane particles of at least about 0.1 mg/ml of therapeutic composition.

11. The therapeutic composition of claim **1**, wherein said therapeutic composition is an injectable composition having a viscosity of no more than about 50 Pa sec and wherein the therapeutic composition can be injected through a 30 gauge or larger needle.

12. The therapeutic composition of claim **1**, wherein said therapeutic composition is a paste having a viscosity of more than about 50 Pa sec.

13. The therapeutic composition of claim **1**, wherein said therapeutic composition comprises a plurality of amnion derived protein markers including basic fibroblast growth factors (bFGF), bone morphogenetic protein 2 (bmp-2), bone morphogenic protein 4 (bmp-4), bone morphogenetic protein 7 (bmp-7), bone morphogenic protein 9 (bmp-9), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), platelet-derived growth factor AA (PDGF-AA), platelet growth factor BB (PDGF-BB), platelet-derived growth factor AB PDGF-AB), transforming growth factor beta one (TGF-b1), and vascular endothelial growth factor (VEGF).

14. The therapeutic composition of claim **1**, further comprising a plurality of non-amnion derived viable progenitor cells.

15. The therapeutic composition of claim **1**, further comprising a plurality of viable vascular fraction cells.

16. The therapeutic composition of claim **1**, further comprising a plurality of viable vascular fraction cells from adipose.

17. The therapeutic composition of claim **1**, further comprising preadipocytes, viable mesenchymal stem cells (MSC), and viable endothelial progenitor cells from a stromal vascular fraction.

18. The therapeutic composition of claim **1**, wherein the carrier fluid further comprises a saline solution.

19. The therapeutic composition of claim **1**, wherein the therapeutic composition is a cosmetic composition.

20. The therapeutic composition of claim **1**, wherein the therapeutic composition is provided with a patch comprising a plurality of nano-needles.

21. A therapeutic composition comprising:

a) acellular amniotic membrane particles;

b) a carrier fluid comprising acellular amniotic fluid;

wherein the acellular amniotic membrane particles consist essentially of micronized amniotic membrane;

wherein the therapeutic composition is essentially free of any viable amniotic membrane cells or viable amniotic fluid cells;

wherein the micronized amniotic membrane particles have an average particle size of no more than about 100 μm;

wherein the micronized amniotic membrane particles consist essentially of amnion and are essentially free of chorion; and

wherein the amniotic fluid is a concentrated acellular amniotic fluid, having a concentration of an amnion derived growth factor of at least about 0.1 pg/ml.

22. A process for the formation of a therapeutic composition comprising the steps of:

a) providing an amniotic membrane;

b) micronizing said amniotic membrane to produce a plurality of micronized amniotic membrane particles having an average particle size of no more than about 100 μm;

c) providing an amniotic fluid;

d) decellularizing said amniotic membrane to substantially destroy any viable amniotic membrane cells to produce an acellular amniotic membrane;

e) decellularizing said amniotic fluid to produce a decellularized amniotic fluid having no more than 500,000 intact cells per ml of said decellularized amniotic fluid; and

f) dispersing said micronized particles into said amniotic fluid;

thereby producing a therapeutic composition having essentially no viable amniotic membrane cells or viable amniotic fluid cells.

23. The process of claim **22**, further comprising the step of concentrating the amniotic fluid.

24. The process of claim **23**, wherein the step of concentrating the amniotic fluid comprises at least one of centrifugation or exposure to vacuum.

25. The process of claim **22**, further comprising the steps of:

adding a plurality of non-amnion derived viable cells to the therapeutic composition.

26. The process of claim **22**, further comprising the steps of:

g) adding a plurality of non-amnion derived viable cells to the therapeutic composition;

h) subsequently cryopreserving said therapeutic composition to produce a cryopreserved therapeutic composition; and

i) thawing out said cryopreserved therapeutic composition to produce a thawed therapeutic composition having said plurality of non-amnion derive viable cells.

27. The process of claim **26**, wherein the non-amnion derived viable cells comprise progenitor cells.

28. The process of claim **26**, wherein the non-amnion derived viable cells comprise vascular fraction cells.

29. The process of claim **26**, wherein the non-amnion derived viable cells comprise preadipocytes, mesenchymal stem cells (MSC), and endothelial progenitor cells.

30. A method of topically treating a treatment location comprising the steps of:

a) providing a therapeutic composition comprising:

i) acellular amniotic membrane particles;

ii) a carrier fluid comprising an acellular amniotic fluid;

wherein the acellular amniotic membrane particles consist essentially of micronized amniotic membrane; and

wherein the therapeutic composition is essentially free of any viable amniotic membrane cells or viable amniotic fluid cells;

b) providing a patch comprising a plurality of nano-needles

c) combining the therapeutic composition with the patch;

d. applying the patch to the treatment location;

e) dispensing the therapeutic composition to the treatment location through said plurality of nano-needles to topically treat the treatment location.

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(12) EX PARTE REEXAMINATION CERTIFICATE (11006th)

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(54) **ACELLULAR AMNION DERIVED THERAPEUTIC COMPOSITIONS**

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(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

To view the complete listing of prior art documents cited during the proceeding for Reexamination Control Number 90/013,712, please refer to the USPTO's public Patent Application Information Retrieval (PAIR) system under the Display References tab.

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(57) **ABSTRACT**

Acellular amnion derived therapeutic compositions are described having a number of various compositional embodiments. An acellular amnion derived therapeutic composition has essentially no live or active amniotic stems cells. The amniotic stem cells may be destroyed, and the cells and cell debris may be removed from the acellular amnion derived therapeutic composition. An acellular amnion derived therapeutic composition may comprise micronized amniotic membrane particles, and/or amniotic fluid. An acellular amnion derived therapeutic composition may be a dispersion of micronized amniotic membrane combined with a fluid, such as plasma, saline, amniotic fluid, combinations thereof and the like. An acellular amnion derived therapeutic composition may be combined with a matrix component to form a composite. An acellular amnion derived therapeutic composition may be used in conjunction with a composition comprising viable cells, such as stem cells.

EX PARTE REEXAMINATION CERTIFICATE

NO AMENDMENTS HAVE BEEN MADE TO THE PATENT

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims **1-30** is confirmed.

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